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What Mr Andropov should do for science

Mr Leonid Brezhnev's death, and Mr Yuri Andropov's succession as the fifth holder of his party post since the Revolution, is also an opportunity for reforming the framework of Soviet science.

For the next few days, Mr Yuri Andropov will have a great deal on his mind. His first need, as one recently emerged from the grey uniformity of the Central Committee (Politburo), is to let the world know that he is flesh and blood. Last Monday's funeral oration was a start. Before turning to matters of substance, Mr Andropov will probably also follow other newly appointed national leaders in replacing the holders of posts with which he must work closely by people who are congenial to him. This will for some time be the chief source of interest to those who make a profession of watching the Soviet Union closely. Meanwhile as Andropov must know well enough, the bureaucracy will look after the day to day business for him. Yet eventually his attention must turn to the issues that occupied his predecessor — East-West relations (poor, even with the lifting of the US sanctions against West European allies for alleged transgressions on the Siberian pipeline), the state of the Soviet economy (parlous) and the state of security and the armed services — and the cost thereof. Will Andropov have the wit to escape this arid agenda to tackle the question none of his predecessors since Lenin (Khrushchev may have been a wayward exception) has had time for — the condition of Soviet science? The beneficiaries of such a departure from immediately past precedent would include not merely Andropov himself, his government, the Soviet scientific community and Soviet citizens at large but also the rest of us.

What follows is a recipe for Mr Andropov's inquiry into Soviet science drawn up on the gloomy but realistic assumption that he will have neither the opportunity nor the inclination to follow radical policies on the questions that most sharply divide the Soviet Union from the rest of the world, the important issues of personal liberty within the Soviet state. It is, of course, offensive that Sakharov is still exiled in Gork'i and that Orlov and the rest are still imprisoned. It is also the equivalent of tying one arm of Soviet science behind its back that the freedom of Soviet scientists to travel abroad is regarded not as an intellectual necessity but as a reward for loyalty, distinction and longevity. (Obituaries in *Pravda* regularly list a deceased's visits to conferences overseas among the other honours that may have been showered on him.) But nobody should expect that these practices can be changed overnight. The supposition that the state can order the lives of ordinary citizens is a hangover from the feudal doctrine that citizens are literally servants of the state. To acknowledge this is not to condone illiberality but merely to acknowledge that even the most enlightened Andropov would have problems on his hands.

Education

Andropov must first pay attention to the educational system, in many ways the best in the world but also the most cumbersome. Primary and secondary education are universal and state-supported. In the decades since the Second World War, Soviet

schools have been acclaimed on several counts, not least for the attention that they pay to the teaching of mathematics, science and technology. In the post-Sputnik years, when curriculum developers in the West were looking for ways of improving the practice of teaching school, they found a great deal of help in earlier Soviet innovations. Yet in the Soviet Union as elsewhere, the overriding difficulties are not those of providing buildings or even schools but of recruiting able teachers. The device of segregating pre-teen students with obvious ability into special schools has demographic advantages but runs the risk, especially with the increasing social stratification of Soviet society, that, as in capitalist Britain, the best teachers will be found teaching the sons and daughters of the middle classes. National olympiads in mathematics and the like, coupled with the conviction of village peasants that a bright young person winning a place in some distant special school is a feather in the local community's cap goes some way to redress the balance, but not nearly far enough. Teaching less, for less time, would be a more equitable but also a more beneficial strategy.

Specialization

Specialization is a more serious problem, especially in tertiary education. Seriously-minded people embarking on a university career cannot even briefly enjoy the sense that the world will soon become their oyster. First degrees take far too long — four or five years is about par for the course. Thereafter, a person might hope to become a *Candidate* in his early thirties and, provided his work is not inconstant, a *Doctor* (of science, or of something else) early in his or her forties. The academic ladder is, as it seems from outside, a device for making sure that sparks of creativity are snuffed out — and for reinforcing respect for the gerontocracy. In the circumstances, the wonder is not that Soviet science tends to be derivative but that so many people have survived the system. Andropov could do worse than begin by asking for an explanation of why the system should be as it is. And why it should not be different.

The organization of Soviet laboratories is another problem. Contrary to naive supposition in the West, Soviet public administration suffers not from being excessively authoritarian but from too meticulous a division of responsibility. Even in the Red Army, military commanders are coupled with political bosses (of whom Mr Brezhnev was once one). Soviet research laboratories universally have two heads, one a scientist, one a commissar. The obvious consequence, that laboratory directors are usually less adventurous than they would be elsewhere, is written in the unglamorous record of recent decades. Paradoxically, this same system seems to allow wild eccentricity occasionally to flourish. T.D. Lysenko's influence in the 1950s would not have been as disastrous if there had not been a succession of laboratory commissars to vouch for his ideological correctness. Andropov should tackle the problem of how the state laboratories are run, and urgently.

Andropov should also worry about the Soviet Academy of Sciences, whose president, A.P. Aleksandrov, was given a place of honour at Monday's funeral orations (after the Minister of Defence, before the ritual worker). Aleksandrov was also a member of the committee that planned the funeral, but inevitably so. The trouble, however, with the academy is that its formal

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autonomy (academicians elect each other) conflicts with its function as an instrument of the state, with responsibility for administering public laboratories and, within the past year, committed to even more direct responsibility for running industrial research and development. The most obvious defect of this arrangement is that it is too reasonable, snuffing out the inevitable tension there must be between those with an interest in discovery (which is the basis of future industry) and those with an interest in current production.

The problem of the academy, unfortunately for Andropov, is not the last that calls for his attention. As in Britain, but more scandalously, there is too great a separation between civil and defence research. Only in the past few years has the Soviet space programme begun to make a bridge between the strictly secret activities of the army of defence researchers and those who serve the academy's institutes, the universities and the generality of public service. Socially and in economic terms, the relatively lowly status of the Academy of Medical Science (whose president N.N. Blotkin spoke after Aleksandrov at the funeral orations) may be unimportant, but the neglect of the Academy of Agricultural Science (whose president did not speak at all) cannot be irrelevant to Andropov's problems. Is it not time (and would it not be politically convenient) that Soviet citizens should for once be fed decently? Mr Andropov might, without disadvantage, ask the academy whose president was so much honoured on Monday why agricultural research has been so foolishly neglected. He may discover that he, like other comparable leaders, needs a mechanism by which loyal but free-minded scientists can tell him how resources should best be spent.

Unsolicited advice such as this is never welcome and often goes unread, not least by those to whom it is addressed. Others may ask why advice intended to be helpful (but likely to be overlooked) is proffered at all, in the present climate of East-West relations. There are two simple answers, which the West too often overlook. First, if the struggle (an Eastern word) between East and West is ideological, and if nuclear warfare can be avoided, engagement is preferable to detachment. Second, as both sides are fond of saying, science is international. So must not even the West be deprived by the diseconomies of Soviet science? From Artsimovich to Zeldovich, the West has profited from those the Soviet system has allowed to flourish: how much has it lost because so many others have been stifled?

West Germany's millstone

The new West German government seems ill prepared to tackle the problems of its universities.

The long-standing legend of the free-floating German student, moving as the fancy takes him from one seat of learning to another, is appealing but no longer a joke. The West German academic system is at serious risk of being crippled by its students, not merely by the huge numbers of them but by their disinclination to distribute themselves among West German universities according to the distribution of qualified teachers. The result is that some places are overwhelmed with students and that others are half empty. In the circumstances, the vision among academic administrators that the impending decline in the teenage population will bring relief is almost certainly an illusion; more probably, the crowded universities will be just as crowded as at present, and the others even emptier.

Everybody knows that freedom of access to higher education is deeply valued in West Germany. It is also understandable that the Social Democrat government, which helped to institutionalize the doctrine of free access with a sequence of legislation going back to 1969, could hardly have set about devising some scheme for telling would-be students where they should study. Even the introduction of the *numerus clausus* in fields such as medicine was regarded by many of its supporters as a betrayal. The new government is not bound by earlier promises, but seems no more willing to make a fight for commonsense. Will it be different after the March elections?

Ambition running cold?

Last week's meeting of the European Science Foundation was a needless disappointment.

The European Science Foundation is a remarkable organization, not a consortium of governments but an organization of publicly supported research agencies and learned academies which have agreed to contribute small (for them) sums of money to common good causes. At the outset, in 1974, there was nevertheless some reason for hoping that this shoestring organization would so command the loyalty of its customers (mostly working scientists), the fidelity of its members and the respect of their sponsors that it would have a chance of growing into an amalgam of a European academy and a grant-making organization. For the time being, however, that prospect had become too distant for comfort and good sense. Last week's general assembly (see page 207) was a poor augury for the future. The foundation has always been careful not to boast about its potential for the future, saying publicly that it had no ambitions to be more than a marginal institution. The message was appealing when it could be disbelieved. What has happened is that the foundation has begun behaving as if it were indeed a marginal institution. Why has this happened?

The temptation is to suppose that the shortage of money that now afflicts the members of the foundation has made them sour, but that is only half the truth. Domestic budgets are an excuse for parsimony, but not the cause of it. The underlying trouble, and the mainspring of the sourness apparently running through last week's meeting in Strasbourg is that the member organizations have become more inward-looking than they were nearly a decade ago. If that were not the case, they would surely have acknowledged the logic of their own laments at the prospect that good causes such as the fellowships and summer-school programme in brain and behaviour research will founder unless somebody finds another sponsor within two years, and have recognized that they had best dig deeper into their own pockets. If such a programme is acknowledged to be worthwhile, and if those who now contribute towards its cost through the European Science Foundation are to be believed when they say that they would contribute to the same programme under a different umbrella, why can they not see that they would be as well served, and the foundation in the process strengthened, if they made Strasbourg the channel for their largesse? The snag seems to be that the mood has changed. A decade ago, people were looking for pan-European institutions. Now they are more conscious of the respect that they can earn on the international scene by being themselves the sources of the small sums of money flowing into international enterprises.

The foundation should nevertheless not despair. Some of its difficulties are procedural — its own cumbersome ways of conducting business, for example, and constitutional problems among its member states, such as that which prevents Austria from contributing honest schillings to other people's running costs. The first of these could and should be removed by means such as those accepted last week, the second suggests that a treaty between governments might after all be preferable to the present clubbiness. The foundation should also seize on the opportunities that now abound for providing the scientific community with an understanding of the changes that are taking place within it. Even relatively simple questions, such as the amounts of money spent on different kinds of science in different countries (once tackled systematically by the Organization for Economic Cooperation and Development) are now neglected. The changing demography of the academic profession throughout Europe, practical questions of the employment of scientists in different states or of the proper relationship between academic research laboratories and industry (the catchword "technology-transfer" applies) could with advantage be taken up. If European organizations are for the time being short of European spirit, the foundation had better find a bread-and-butter role to keep itself alive until the spirit waxes again.

Stanford's hope for heavy boson

Linear machine may give an edge on CERN

Washington

Physicists at the Stanford Linear Accelerator Center are keeping their fingers crossed during the next few weeks while the Office of Management and Budget (OMB) decides on their \$44 million request to begin construction of the Stanford Linear Collider (SLC) in fiscal year 1984. Approval would put SLC in the running with CERN's large electron-positron storage ring (LEP) to be the first machine to produce the roughly 90 GeV electron-positron collisions needed to generate the elusive Z^0 particle.

"If we get that funding," says Burton Richter, director of the SLC project, "we would be ready to turn on in October 1986". The \$44 million would be the first instalment of a total of \$110 million required for construction on a three-year schedule. LEP is scheduled for completion in late 1987.

Richter admits that the possibility of beating CERN to the punch has helped fuel enthusiasm for SLC. That enthusiasm seems to carry through the physics community and the Department of Energy's high-energy physics office, a broad base of support that may help the proposal over the twin hurdles of OMB and Congress. OMB's decision will not be made until December, and will not be made public until the President's 1984 budget is released at the end of January. At present, though, indications are that SLC may get the go-ahead. Earlier this year, the Energy Department's High Energy Physics Advisory Panel (HEPAP) strongly endorsed the project, recommending a 1984 start.

William Wallenmeyer, who directs the Energy Department's high-energy physics programme, points out that SLC has already rated "rather high priority"; the 1983 budget provides about \$10 million for research and development in connection with the project.

But in its closed-door deliberations, OMB may find several reasons to delay construction. Chief among them is that the physics aspect of SLC — namely the ability to study the Z^0 — is only a part of the rationale behind the project. Wallenmeyer says the "primary justification" is that SLC would allow a new concept in accelerator design, called the "colliding linac", to be tested. This new breed may prove to be a less expensive alternative to the circular accelerators.

Thus a modest delay in SLC would not

harm this primary goal. SLC would be built by adding a loop at the end of Stanford's existing linear electron accelerator. Electrons and positrons would be accelerated down the linear stretch; the electrons would then follow one branch of the loop, positrons the other, and they would collide at the far side of the loop. Energies of up to 50 GeV per beam (100 GeV in the centre of mass in the collision) are anticipated.

Another possible counter to Stanford's push for a 1984 start and a quick three-year construction schedule is that the physics that can actually be done on SLC may in fact be rather limited. George Trilling of Lawrence Berkeley Laboratory chaired a HEPAP subpanel that last January recommended that SLC go ahead in 1984 even under a "low-budget" option — with the savings to come from a cut-back in Brookhaven's ISABELLE accelerator project; yet Trilling says that SLC is "not anywhere on the scale of LEP or ISABELLE; it's of a more limited scope". LEP, he says, will be of general use to high-energy physicists, while the physics on SLC will be limited to the Z^0 work. And Wallenmeyer points out that the actual discovery of the Z^0 is likely to come from an existing machine at CERN, which collides proton and antiproton beams. "The purpose of LEP or SLC would be to pursue the resultant physics that comes out of that", Wallenmeyer says. The electron-positron

collisions in these machines make the interpretation of decay products much simpler.

None of this, however, discourages Richter in his role as director and chief proponent of SLC. Although SLC will have a single detector as opposed to LEP's four, he says, "SLC has as much physics potential as LEP." Furthermore, he says, SLC will provide a smaller beam (1.4 μm radius compared with LEP's $500 \times 50 \mu\text{m}$ cross-section), making the decay of short-lived particles easier to study; SLC will also be able to generate longitudinally-polarized beams, a boon in attaining high precision in weak-interaction experiments.

Richter also discounts the significance of LEP's higher energy capability. In its current design configuration, LEP will produce energies up to 80 GeV per beam; going beyond that, Richter says, would require a new technology as yet untested. Richter says that once the Z^0 resonance is reached at what is predicted to be 94 GeV (47 GeV per beam), there is no interesting physics until about 200 GeV (100 GeV per beam), when pairs of charged W^+ / W^- particles would begin to be produced in significant quantity.

For all of the build-up, the race between LEP and SLC may be determined in the starting gate if OMB decides to postpone construction of SLC. But if SLC gets the go-ahead for a 1984 start and a three-year construction schedule, it may be a race down to the wire.

Stephen Budiansky

CERN unafraid of Stanford

Undismayed by the news from Stanford (see accompanying story), the European centre for nuclear research (CERN) is still hoping that it will find the heavy boson in the next few months.

Over the past 4 weeks, CERN's system for colliding protons and antiprotons with each other has been working much better than before. "We are now really in business", said CERN director Professor Herwig Schopper last week. The collisions should produce both the charged intermediate vector bosons, the W^+ and W^- , and the neutral one, the Z^0 . The collider is working well enough to produce "a few" bosons before Christmas, although it will be difficult to distinguish them from the many other particles produced in the high-energy collisions. The interest in the particles is that their existence is a clear prediction of presently widely accepted unified field theories, so that their detection will provide a critical test of the theories.

Meanwhile, contracts are now being prepared for around a third of the components for the construction of the large electron-positron collider, LEP, which is CERN's next major project. LEP is

designed to give the clearest possible investigation of intermediate boson and related physics. The first contracts to be agreed have been for a major section of the 27-km tunnel, for the production of 3.6 million magnet laminations and for steel and concrete cores for the magnets. They have gone to the lowest bidders, which have been consortia involving Italian, French, German, Swiss and Austrian companies. The prices have been roughly as planned, and certainly not low enough, says Schopper, to bring forward the completion date of late 1989.

Construction will not, however, begin until the French authorities give their environmental seal of approval. Two-thirds of LEP lies in France, and one affected village, Echevenex, has made a particularly strong protest (see *Nature* 30 September, p.385). The local review procedure is now over, and CERN must now make its own representations to the committee. CERN has two possible new LEP designs for the Echevenex region. The committee will submit its recommendation to the French government which is expected to come to a decision early next year.

Robert Walgate

European/Soviet collaboration

Doubts surface

The Committee of the Council of the European Laboratory for Particle Physics (CERN) has failed to reach an agreement on the proposed renewal of its collaboration with the Soviet Union. The Soviet side is anxious for this collaboration to be extended, particularly in view of the construction of CERN's new electron-positron collider (LEP).

The lack of agreement among the committee about the renewal reflected the continuing concern about human rights in the Soviet Union, in particular the continuing repression of such "eminent scientists" as Dr Yuri Orlov and Academician Andrei Sakharov. Since 1978, when Orlov received a 12-year sentence, CERN has taken a special interest in his fate.

The CERN delegates reflect the differing views in the West on how far the human rights issue should be linked to decisions on scientific and trade cooperation. The United Kingdom delegate, for example, expressed a belief in the importance of collaboration in science, saying that Soviet participation in LEP experiments "would be of concrete benefit to the international scientific community", although admitting to concern at the "present record of the Soviet Union in relation to human rights".

The Italian delegation, however, felt that such a sensitive issue should be settled only with the approval of the wider scientific community. They urged, therefore, that a new cooperation protocol be drawn up for ratification by the CERN Council, whose proceedings are public, rather than simply by the committee in closed session.

The machinations of the CERN committee were undoubtedly influenced by the imminence of the Madrid Review Conference of the Helsinki Act, which reopened on 10 November. The CERN Orlov Committee was a co-sponsor, with the *Comités Scientifiques Françaises* and "Scientists for Sakharov, Orlov and Shcharanskii" (United States), of a special press conference in Madrid, designed to draw attention to the plight of their Soviet colleagues.

The death of Leonid Brezhnev, and the accession to the post of General Secretary of the Communist Party of the Soviet Union of Yuri Andropov will inevitably evoke considerable rethinking of East-West scientific exchange. Although in his funeral oration on Monday, Mr Andropov expressed a commitment to detente and a willingness to cooperate with any country which genuinely sought cooperation, he also pledged support to the "strengthening" of socialist government at home and abroad, which could well lead to actions unacceptable to human rights lobbies in the West.

Vera Rich

French science research

Papon explains his plans

The Centre National de la Recherche Scientifique (CNRS), the research council which dominates basic science in France, seems to have set off on the right foot with its new director-general, Professor Pierre Papon, who came to the post hot-foot from a political position at the ministry of research and industry in October.

Initially, some feared that Papon's close links with the ministry would increase political influence over fundamental research policy — but his first statement of position to the CNRS council a few days ago confirmed the continuity of his thinking with that of his predecessor, Professor Jean-Jacques Payan, and even hinted that CNRS may influence the government, rather than the reverse.

Papon explained that he had four main objectives to achieve: to create "a new dynamics" in CNRS science; to "open" CNRS to cultural, economic and social needs, and towards the international community and the Third World; to "democratize" decision-making; and to make CNRS "one of the main partners" of the government in the whole process of national science policy-making.

By "new dynamics" Papon means to create new areas of research and to stimulate others — such as general microbiology, or the neurosciences — which are not considered to be at a sufficiently high level in France. Also French science is often strictly compartmentalized, and Papon intends to help break these barriers and induce the movement of ideas and people by setting up new interdisciplinary programmes, such as the programmes on drugs

(PIRMED) and materials (PIRMAT).

Papon also wants to loosen the distinction between pure and applied research — although, he insists, "of course CNRS must remain 80 per cent basic". His guiding light is Jean-Frédéric Joliot, the Nobel-laureate radiochemist who was the first director of CNRS in 1944–45. "Joliot thought of applications of radioactivity in the 1930s, when Rutherford was dismissing it as a joke" said Papon. Joliot, although a basic scientist, took out early patents on a primitive radioactive pile. He demonstrated that the pursuit of truth and profit are not incompatible.

As for "opening" CNRS to the outside world, Papon will create two new directorates, one to pursue the application of CNRS results in industry, and one to deal with information, publicity and international relations. Papon, a socialist, stresses his own interest in improving CNRS links with developing countries. But his policy here will be guided by French foreign policy.

The third line of Papon's policy, "democratization", he interprets as "complete transparency": decisions will still be taken by the directorate, but in the open, after wide debate.

The fourth line, making CNRS a partner in government policy-making, means essentially that Papon, with his strong political links and experience, will not hesitate to make positive science political proposals to the minister of research and industry — and other ministers. There will be no waiting meekly for the directives to come from above.

On other issues, Papon is convinced of the value of the 12-year-rule (see *Nature* 14 October, p.569, and 21 October, p.670) by which laboratory directors will have to resign their posts after 12 years in the job; and believes that Dr Maurice Godelier, an anthropologist who a year ago was bitterly opposed as potential director for the social sciences at CNRS, is now broadly acceptable to the social science community "as far as I can tell". Godelier's report on the social sciences in France (see *Nature* 28 October, p.771) is "a masterpiece", according to Papon, and generally agreed to be a good assessment. The appointment of a director for social science and humanities should be made in a few weeks, and although Papon would be happy to work with Godelier he would also accept "any open-minded person" in the job.

On the 12-year-rule, Papon believes it helps to know you have a finite time in which something concrete must be achieved. The only problem is with the smaller groups, set up around one scientist whose ability has been the *raison d'être* of the team. But the rule, as later qualified by the research and industry minister, leaves room for manoeuvre.

Robert Walgate

Good news for BAS

The Falklands War seems to have worked to the advantage of the British Antarctic Survey. The Department of Education and Science announced last week that it is to allocate an extra £4 million in 1983–84 in addition to the normal allocation expected from the Natural Environment Research Council (NERC). After a long period of tight budgets, this appears to be the beginning of an expanded commitment by the British towards Antarctic research. NERC's allocation to the survey for this year, £7.8 million, is already significantly more than last year's £5.6 million.

No reason was given for last week's announcement, and it is not yet known how the extra money will be spent. The Falkland Islands are used as a refuelling and supply base for Antarctic survey support ships. At present there is a numerically superior Argentinian presence in the Antarctic Peninsula, with 158 Argentinians (132 of them military personnel) and 58 British survey scientists.

David Millar

European Science Foundation

Living with level funding

Strasbourg

This year's general assembly of the European Science Foundation (ESF), at the European Parliament on 9–10 November, was curiously disappointing. For almost the first time since its creation in 1974, the foundation spent as much time deciding how and when to bring good projects to an end as on the problems of how best to launch new enterprises.

Part of the trouble is that the foundation seems to have been infected with the present preoccupation of its members — all of them research organizations in the member states (which include Yugoslavia, Turkey and Israel as well as countries in Western Europe and Scandinavia) — the problem of living within a constant budget. In this spirit, the assembly spent much of its energy this year wrestling with the question whether one of its first and most successful programmes, the European Training Programme in Brain and Behaviour Research, should be pushed towards independence one, two or three years hence. But the assembly's consideration of new projects has also been strangely flat and uncritical.

The foundation is for practical purposes a club whose members are publicly supported grant-making agencies in the member states. Each national group pays a collective subscription determined by its home state's Gross National Product, but may then contribute voluntarily to the several "additional activities" that make up the foundation's research programme.

In such circumstances, it is hard to tell how large is the effort subsumed under the umbrella of ESF. This year, for example, central expenditure will amount to FF6.8 million (£0.5 million), but cash contributions for additional activities will amount to FF5.7 million and there seems to be a gentlemen's agreement that overhead costs should be concealed wherever possible. The proposal that central expenditure next year should rise to FF8.1 million — rather less than 0.05 per cent of the budgets of its members — was accepted without a trace of dissent, no doubt in gratitude for the foundation's implicit undertaking that from now on it will take on new projects only when it has discarded others.

The counterweight for the proposed abandonment of the brain and behaviour programme is the European Geotraverse project, a plan to mount a series of deep seismic soundings along a north-south band running from northern Norway to Tunisia, together with coordinated geological, geochemical, geomagnetic and magneto-telluric measurements (see *Nature* 29 July, p.413). The project was enthusiastically endorsed by the assembly, or at least by those of its members whose national boundaries lie near to the traverse.

Enthusiasm does not however mean money. The traverse project will require 14 million Swiss francs (£3.75 million) for central funds, while the planners (led by Dr P. Fricker, secretary-general of the Swiss National Science Foundation) hopes that a further FF8 million will be spent by member states on regional studies. In the event, roughly half the countries concerned were able to commit themselves, in a genteel version of a political fund-raising affair, to contribute to the costs of coordinating the enterprise.

In future, the foundation hopes to have a better mechanism for recruiting contributions towards such additional activities, the assembly having adopted at this meeting a set of proposals negotiated by a group under Dr Fricker that will allow

European space research

Backing away from Ariane?

European astronomers, increasingly concerned about the delays in the Ariane rocket programme, are pleading that the European Space Agency (ESA) should consider launching their next X-ray satellite, called EXOSAT, on a Thor-Delta rocket. A decision to switch launchers at this stage would embarrass (but possibly relieve) Arianespace and cause contractual problems (but probably decrease the cost). According to one senior X-ray astronomer, however, the case for switching launchers is now undeniable on scientific grounds.

The three-stage Ariane rocket has failed, for different reasons, on two out of five of its launches. A committee of inquiry has determined that the failure on the last launch was due to problems with lubrication and design tolerances in the rocket's turbopumps. These are being modified before the next launch in April. On present plans EXOSAT would be launched on the following Ariane in the middle of next year.

EXOSAT, the only X-ray astronomy satellite expected to fly for several years, represents a significant investment over the past few years by European astronomers. Several aspects of its design reflect the recent rapid development of X-ray detectors.

The satellite was originally designed to be launched on a Thor-Delta rocket, and the subsequent decision to change to Ariane had a strong political (chauvinistic) element. Accordingly, only minor structural modification would be necessary to go back to a Thor-Delta launcher, which has an enviable record of successful launching.

Now British, German and Dutch astronomers have written to ESA asking that EXOSAT be an urgent item for discussion at the December meeting of the

interested members' budget contributions to be negotiated while projects are being developed. As things are, members have no cause to dissent in public from proposals they consider to be half-baked, knowing that silent assent need cost them nothing.

For the rest, the assembly reappointed Professor Herbert Curien as president for a further three-year term, heard Sir Hermann Bondi plead for a better understanding and appraisal of environmental problems, agreed that something should be done to improve legislation covering archaeological sites, agreed that similar laws are needed to secure meteorites for science, applauded those of its members seeking to persuade some European government to play host to a European source of synchrotron radiation and approved a plan to hold an interdisciplinary meeting on the criteria for progress in science in whose specification the word "discovery" does not appear. ●

Science Programme Committee and requesting that "all possibilities be considered". Any recommendation made by that committee would have to be ratified by the council of ESA, which would normally place much weight on the programme committee's recommendations.

A Thor-Delta would cost about 10 million accounting units (AU) — about £5.4 million at current prices — less than Ariane but ESA has already paid Arianespace 30 million AU for the EXOSAT launch. Arianespace might, however, be relieved not to have to launch EXOSAT. First, it is under pressure from commercial users to launch their satellites on schedule. Second, the EXOSAT launch would require a hitherto untested fourth stage to be added to Ariane, leading to the increased possibility of another failure.

There are other technical reasons why X-ray astronomers would push hard for a switch. Thus it is possible that a Thor-Delta could be ready in time to launch EXOSAT at the end of the current window. (EXOSAT's orbit is highly elliptical, permitting more extended observations of variable sources and more continuous contact with ground tracking stations than with previous X-ray satellites. The "windows" are those periods in the year during which a launch would provide the appropriate combination of solar cell illumination and the correct location of the orbital apogee in the sky.) With Ariane, this window ends in January, and another launch would not be possible before the middle of next year. The use of a Thor-Rocket, obviating the need of Ariane's transfer orbit, would extend the window to February, giving the possibility of an "early" launch and the avoidance of six months' degeneration of X-ray detectors stored in laboratories.

Philip Campbell

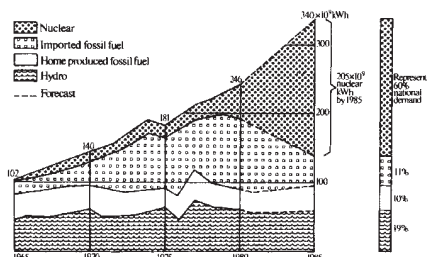
French nuclear power

Testing time for reactors

The problem with French nuclear power stations is that three-quarters of them are less than three years old, and suffering from teething problems, according to Electricité de France (EDF), which owns and runs them.

What is not clear is whether this should be a comforting or a disquieting thought. French nuclear power production is supposed to be increasing, but in September EDF produced only 6,700 GWh of electrical energy, compared with 8,200 GWh a few months previously. The net availability — the proportion of energy delivered to nominal capacity — fell to below 50 per cent in the summer, compared with a typical industry target of 70 per cent and a world average for pressurized water reactors (PWRs) (according to EDF) of 60 per cent. But, says EDF, PWRs more than three years old had the magic 70 per cent availability. It was the young ones — an independent design series of 20 or 50 — that were in trouble.

The accompanying figure reveals how much of the French nuclear power



Power consumption in France

programme is indeed young, coming on line during 1980–85. This is literally a testing time for French nuclear power. Of the 22 reactors now connected to the grid, only 6 were connected before 1980. Seven were connected that year, seven in 1981, and two have been connected this year. However, another 30 are under construction, due to come on line during 1983–89.

According to EDF, the problems amount to a combination of technical teething troubles and government safety regulations. There are three issues: a concentration of seven four-month shut-downs for obligatory inspections, after 18 months' operation, compared with four such shut-downs in 1981 and two planned for 1983; and two problems with faulty materials in the younger reactors.

One of these problems is outside the nuclear part of the reactor, in the steam "drier-superheater". This halted two reactors, Saint-Laurent B1 and B2, for a considerable period, but the problem is now thought to have been solved for these reactors and others in their production series.

The more awkward problem is the stress-corrosion cracking of inconel clips holding

guide tubes for the control rods within the reactor vessel. The problem was first noted in Japan in 1979, and since then the clips have undergone redesign in French reactors beginning construction. But most reactors coming on line in the past three years have the old clips, and there have been failures and shut-downs this year at Fessenheim 1, Bugey 2 and 4 and Gravelines 1. The clips are to be replaced on a series of 20 reactors, at a net cost, according to EDF, of about FF 1,300 million (£110 million).

But is this the end of the "teething" troubles — or will there be more to come, when the problem, like the clips and the

US defence

Townes in dissent on MX siting

Washington

Controversy continues about how and where the new MX missiles should be based. President Reagan is committed to give Congress a plan for basing the MX (short for "missile experimental") by 1 December and the Secretary of Defense, Mr Caspar Weinberger, has done his part by transmitting his recommendations to the White House, apparently on 1 November. But now his most prominent scientific adviser on the issue, Dr Charles Townes of the University of California at Berkeley, has taken the unusual step of expressing his personal doubts about the now-favourite plan — and the contents of his letter have been promptly leaked in the US press.

The question of how to deploy MX has been a long-standing conundrum. Out of about 30 alternative plans suggested by the military, the Carter Administration selected that called "Racetrack" in which missiles would have been moved from one launching point to another by means of underground tunnels. But by the time the plan was put forward, so many doubts had arisen that Congress never approved it.

The same fate may yet await the Reagan Administration, which has already failed to persuade Congress to authorize funds for deployment — without specifying how the missiles would be deployed. Townes was appointed head of a panel of scientists and defence experts to re-examine the MX problem when Mr Weinberger took office. The scheme now most favoured by the US Air Force and, it is rumoured, by Mr Weinberger is called "Dense Pack". MX missiles would be placed in fixed and hardened canisters in the ground so close together that incoming warheads would interfere with each other, the blast and other consequences of a nuclear explosion interfering with later arrivals.

The Air Force now says that 100 MX missiles separated by distances of about 2,000 feet would be close enough to ensure

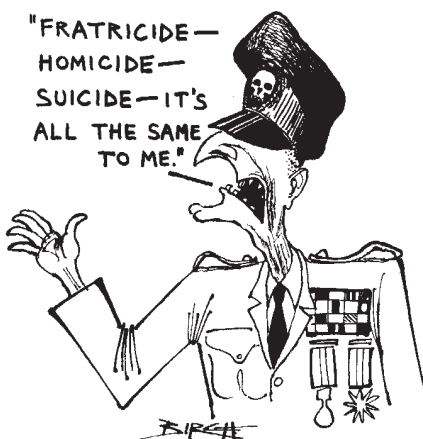
drier-superheaters, will be multiplied through a long production series leading to high replacement costs, and a degree of insecurity in future electricity supply, which is planned to reach 60 per cent nuclear by 1985?

However, this can be taken as an argument for building more nuclear reactors, not less, to provide a cushion of overcapacity, and it will no doubt be taken that way at the ministry of research and industry in the forthcoming argument over coal and nuclear power and falling energy demand forecasts (see *Nature* 11 November, p. 100). In Britain, too, where the Central Electricity Generating Board is planning its first PWR at Sizewell, Suffolk, the French problems are seen in a positive light — as useful design lessons. **Robert Walgate**

interference (called "fratricide" in the new jargon). Hitherto, the Air Force has discarded Dense Pack on the grounds that missiles not destroyed in an attack would be unable to fly out in retaliation. The new development is that the Air Force says that missile silos can be hardened so that their missiles will survive even if over-pressures amount to 5,000 lb per square inch.

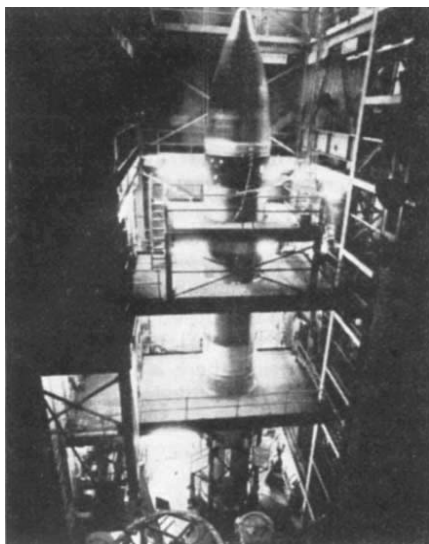
The nub of Townes's argument is that this "super-hardening" may not be feasible, that there are no clear limits on the time that would be needed for such a development or of its cost and that the Soviet Union might find a counter to Dense Pack sooner than the Air Force could install it.

Military considerations apart, Dense Pack has the political advantage that it would occupy an area of only 10–15 square miles. One of the objections to Racetrack from ranchers and environmentalists in western states was that huge tracts of land would be required.



The latest version of Dense Pack advocated by the Air Force is that the 100 MX missiles would be sited in a long thin column aligned north to south. This has the theoretical advantage that the optimum pattern of an attack on the missile field

from the Soviet Union, called a "walk attack", can be predicted: the most southerly rows of missiles would have to be attacked first and succeeding warheads would have to be aimed at suitable intervals at rows of missile silos sited further north. On this argument, a pattern of attack moving from north to south would be ineffective because later warheads would have to survive the atmospheric disturbance caused by the first nuclear explosion.



It is not clear what the consequences will be for Dr Townes now that his strong expression of personal doubt about the government plan has leaked into the press. Scientific advisers have run into trouble on such grounds on many previous occasions. But Dr Townes is much respected within the present Administration and his opinion may even help the President to reach a wiser decision by the beginning of next month.

Deborah Shapley

Yes to toxin cloning

Washington

The Recombinant DNA Advisory Committee (RAC) approved two controversial toxin-cloning experiments at its 25 October meeting, but ordered that they be carried out under P4 containment, the most stringent safety precaution available.

Dr John Murphy of Harvard Medical School, who in July 1981 received permission to clone the diphtheria toxin gene in *Escherichia coli* K-12, will now be allowed to extend those experiments to linking the toxic gene with the gene that codes for melanocyte stimulating hormone. The aim is to generate a hybrid toxin molecule that selectively homes in on melanoma cells.

The experiments will have to be performed at the National Cancer Institute's laboratory at Frederick, Maryland, which is the only P4 facility in the country. Stephen Budiansky

German research and technology

Waiting for the next election

Bonn

Dr Heinz Riesenhuber, appointed last month as West German federal minister for research and technology, seems to have made a good first impression. But it is also clear that between now and the promised general election next March, there will be time only for some modest if urgent house-keeping, and for planning how to put flesh on the bones of the promises of the past few weeks.

The federal ministry for research and technology has two immediate tasks — to win agreement on a budget for next year (and on a supplementary budget for 1982) within the caretaker government and with the parliament; and to put the two big prototype nuclear reactor projects on a sound financial basis. Both issues must be settled before the year is out, for thereafter all contentious issues will be overtaken by preparations for the election.

The budgetary and reactor issues are closely linked, for the reactor construction costs are a large part of the federal ministry's budget. There seems a good chance that the parliament (Bundestag and Bundesrat) will fall in with the previous government's proposal that the ministry should have a supplementary subvention of DM500 million (£116 million) with which, for practical purposes, to liquidate bank loans taken out by participating electricity utilities to keep construction going. (The propriety of this device could nevertheless become a political issue at the election.) If the Christian Democrat government is re-elected, Dr Riesenhuber's belief that both reactors should be completed will nevertheless become a simplifying touchstone for future policy: although, as one high official emphasized last week, the government will not support the projects "at any price". But the new government seems to understand that new technology is never more expensive than when it is postponed.

The implications of Dr Riesenhuber's first-flush declaration that research and technology must share in the new government's commitments to a six per cent reduction of spending, but that "basic research" must at the same time be protected, are harder to discern. Some officials hope to find some relief from the strictest possible application of the six per cent rule in the *caveat* that it may not apply when the consequences would be legally invalid. Most of the ministry's projects entail agreements with constitutionally autonomous Länder governments for example, and may therefore be exempt. The budget for 1983 now being canvassed in the Bundestag, at just under DM7,000 million, is a reduction of DM300 million on the original budget for 1982 — the first reduction since the ministry's creation in 1971. But it seems to be an article of faith

that when economies are decided, they will not affect basic research.

Dr Riesenhuber's other memorable declaration, that the large national research laboratories should have a closer relationship with industry and that direct support for research and development should progressively be replaced by "indirect support", is for the time being equally uncertain in its implications. The minister's conviction that civil servants cannot adequately supervise the more than 6,000 direct-support projects now on his department's books is readily accepted, but nobody has a clear idea of what indirect support consists of. The notion that the federal government might subsidize the salaries of qualified scientists and engineers working for small and medium-sized companies is one being canvassed. By the general election, Dr Riesenhuber may have other cards up his sleeve. Meanwhile, he is committed to the award of a prize of DM30,000 at some point during 1983 to somebody working in a public laboratory making the most outstanding contribution to industry.

No doubt in the hope of avoiding similar problems with thermonuclear fusion, the federal ministry of research is planning to peg its total expenditure on fusion, principally at the Max Planck Institute at Garshing and the JET laboratory at Culham in the United Kingdom, to about DM200 million a year. It has been decided that Garshing will be concerned only with plasma physics machines, and that the nuclear research establishment at Karlsruhe will assume responsibility for any experiments in which thermonuclear fuel is consumed to produce radioactive materials.

Elsewhere, the prospect that a change of government may stimulate other changes seems now, as ever, frustrated by the constitution. Nobody thinks it feasible that the excess numbers of students at the most popular universities could be limited by some selection process, or even arbitrarily; the Länder would not stand for that. The federal ministry of education is nevertheless hoping to help cure the enforced immobility in academic life in the past few years with a proposal to spend DM50 million a year on the appointment of up to 1,000 newly graduated PhDs to one- or two-year posts in universities. This development, valuable in itself, is also a test of the federal government's undivided constitutional responsibility for research. The bill now before the Bundestag does not depend on the compliance of the Länder governments.

The education ministry, following the doctrine that basic research should be protected, has also managed to increase the budget of the Deutsche Forschungsgemeinschaft for next year by four per cent.

European Molecular Biology Laboratory

Crucial decisions ahead

Heidelberg

This month is crucial for the European Molecular Biology Laboratory, supported since 1974 by eleven European governmental research agencies, a subset of the seventeen members of the European Molecular Biology Organization. Next week the governing council of the laboratory will decide whether it can match with deutschmarks its acceptance of a new scientific programme at its meeting last June. The director of the laboratory since 1 April, Dr Lennart Philipson, is dismayed by suggestions such as that in the report of the British Advisory Board for the Research Councils (see *Nature* 4 November, p.1) that the Medical Research Council should "review" its membership of the laboratory.

The Medical Research Council refuses to comment on this recommendation. It is however known that its secretary, Sir James Gowans, paid an unofficial visit to the laboratory in September, since when there have been two visits by parties of officials to the laboratory and its two outstations at Hamburg and Grenoble.

Whatever the UK research council's attitude next week, the council meeting is certain to be uncomfortable. Philipson is asking for budget contributions of DM36.7 million (£8.5 million) for 1983, a substantial increase on the total contribution of DM 30.2 million in the present year. Philipson emphasizes, however, that a direct comparison of these figures is misleading. In past years, the laboratory has consistently failed to recruit up to its planned strength, with the result that there has usually been a cushion of unspent appropriations to help with the succeeding year's budget. During the present year, members' contributions have been augmented by more than DM6 million from this source, but for next year there will be a cushion of only DM2 million.

The perplexing question now is whether next week's council will accept that the planned increase in expenditure amounts only to 6 per cent, or whether it will take fright at being asked for a 20 per cent increase in members' contributions.

The more distant but more important question of what the laboratory is for has, however, been settled by the scientific plan agreed in June. The laboratory now accepts that there is no means by which its existence can be justified on grounds comparable with those applicable to international laboratories such as CERN in Geneva. While the synchrotron radiation and neutron diffraction facilities (at Hamburg and Grenoble respectively) are much used, especially by workers from smaller countries, they are not unique. And the prospect that the low-temperature electron microscope developed at the laboratory might be a focus for inter-

national collaboration has receded as the predicted resolution of that instrument has decreased (see *Nature* 30 September, p.386).

The new programme entails that the instrumentation division at the laboratory will become a source of new technology in molecular biology for the laboratory and member organizations. The development of the low-temperature electron microscopes will continue, but the excellent workshops will be increasingly used for building prototypes of instruments for which there is a known need.

In molecular biology proper, the laboratory is best known for its work on membrane proteins. On the basis of workshops held earlier this year, it is now planned also to concentrate on two new fields — protein-DNA interactions (as in the functioning of regulatory nuclear proteins) and differentiation (as typified by the haematopoietic system. Dr Thomas Graf has been recruited from the nearby German Centre for Cancer Research (on a five-year rolling contract) to help develop the second line of inquiry. Meanwhile, the structural group at the laboratory will concentrate on emerging opportunities for

the structural analysis of membrane proteins.

The long-term objective is thus to create a recognized centre of excellence, but also more fully to implement the laboratory's brief as a training centre. There will be one summer course next year, and a series of courses between June and September in 1984. Meanwhile, the laboratory is arranging with European universities to recruit up to ten graduate students a year.

The development of the scientific programme is the source of Philipson's immediate difficulties. The laboratory's target payroll of 267 people (excluding visitors) has never been filled, but next year's budget allows for 255 people, a substantial increase on this year. There is also to be a system of external reviews of scientific programmes at intervals of three years.

It seems to be accepted that in its new form the laboratory will be of more immediate value to member organizations too small to sustain substantial molecular biology laboratories of their own. But Philipson says that there is one sense in which Heidelberg is likely to become unique — it has been laid down that scientists on the payroll should not have external links with commercial organizations, although they may work as consultants during vacation time. ●

Industrial sponsorship

Fewer payrolled students

Industrial sponsorship schemes, under which a favoured proportion of Britain's 251,000 undergraduates receive a substantial financial boost from industry, seems to be on the decline. A better understanding of the trend is likely to come from a new research project to be conducted by the Institute of Manpower Studies (IMS) at the University of Sussex.

In some subject areas, notably electrical engineering, as many as 50 per cent of all undergraduates may be sponsored, according to Richard Pearson, head of the IMS Labour Market Studies Group and director of the study. Yet despite the obvious importance of the practice for graduate recruitment patterns in general, there is a striking absence of hard information about figures and trends. Sponsorship may mean up to an extra £800 per year to an undergraduate, and some employers, such as British Telecom, actually reward their favoured potential recruits with a salary while they are still at college. In return, there is a gentleman's agreement that the student will accept any offer of employment from the sponsoring company after graduating. Of undergraduates who started their courses in 1980, 2,000 at universities and nearly 1,000 at polytechnics found an industrial sponsor, mainly in the fields of electrical and mechanical engineering. The IMS study, which is supported by a £55,000 grant from

the Leverhulme Trust, will concentrate on sponsorship by individual companies in both public and private sectors.

Most of the sponsorship schemes now operating started in the late 1970s and apply to the general area of engineering, where there was until recently a severe shortage of suitably trained graduates. Up to 50 per cent of all students taking sandwich courses (which include one or more periods of training in industry) are thought to receive some form of sponsorship. Dr Clive Purkiss, director of IMS, has drawn attention to the lack of clear information on which employers can base sponsorship decisions. Although industrial sponsorship may be regarded as being partly a public relations gesture by the sponsoring companies, it does enable them to get an inside track in their graduate recruitment programmes. It may also be the cheapest way of ensuring an adequate training in a specialist discipline, which according to one estimate may otherwise cost up to £75,000 to the age of 25.

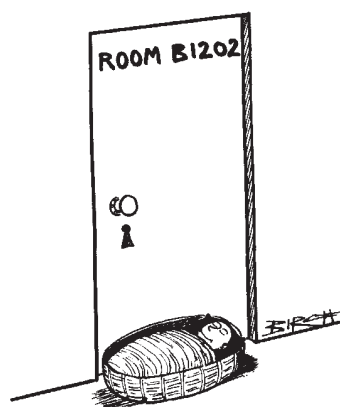
The past two years, however, have seen a drop in the number of sponsored places available: British Telecom's total is down slightly this year to 32, and BP Ltd has just axed its scheme altogether. According to Professor Ray Wild, of Brunel University's Special Engineering Programme, this is partly because companies have become more selective, preferring now to identify

potential recruits before they start a degree course rather than to pick them out part of the way through... The number of students on National Engineering Scholarships (financed jointly by the Department of Education and Science and the Department of Industry) has also been reduced this year, from 300 to around 100. At the same time, educational institutions are trying to increase sponsorship for their students. Some colleges actually specify industrial sponsorship as a requirement for some of their courses, as at London's Imperial College. Students are simply issued with a list of potential sponsors and told to make their own arrangements; the value of the sponsorships on offer varies considerably.

At the moment few small firms sponsor undergraduates, so major employers who run generous schemes can take their pick of the best potential engineers and managers. Less well known firms have to compete against household names in the minds of school sixth-formers who have little knowledge of the industry: Brunel University, whose engineering students are all fully sponsored, is organizing a consortium of several groups of small companies to help overcome this difficulty. The IMS research should receive widespread attention, not least from the students themselves. **Tim Beardsley**

Warnock seeks evidence

The chairman of the British government's inquiry into human fertilization, Mrs Mary Warnock, has asked for all interested bodies and individuals to submit evidence before the deadline of 1 March 1983. The enquiry, due to report in 1984 has a broad brief — covering social, ethical,



legal and medical aspects of developments in human fertilization including *in vitro* fertilization techniques. Evidence should be submitted to: The Secretary, Inquiry into Human Fertilization and Embryology, Room B1202, Department of Health and Social Security, Alexander Fleming House, London SE1.

UK graduate employment

No jobs for the boys (or girls)

About 15 per cent of this year's crop of first degree graduates from British universities will still be looking for jobs at the end of the year. This gloomy estimate was made by Mr B.E. Steptoe, chairman of the statistics subcommittee of the Association of Graduate Careers Advisory Services; 11.3 per cent of that year's graduates had not found jobs by the end of December. Among polytechnic graduates, the figure is likely to be even worse.

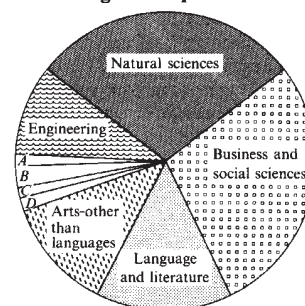
The position of this year's 105,300 new graduates is, of course, made worse by the fact that they are now competing for jobs with those of the 1981 crop still unemployed. As a result, many companies that previously recruited graduates straight from college are now looking for people with some useful work experience. There are also increasing numbers of "under-employed" graduates doing jobs that previously would not have required a degree; some of these will undoubtedly find more suitable employment in time, but a large proportion are likely to remain under-employed.

The trend is described by Mr Brian Putt, director of the Central Services Unit (an advisory body to graduate careers services) as a "disastrous social iceberg". The number of graduates entering the job market will continue to rise by about 2-3 per cent each year at least until 1984, when the effects of last year's cutbacks in higher education places will become apparent.

Unemployment continues to be highest amongst those reading arts subjects, and of the sciences biology clearly has the largest proportion unemployed. However, factors

such as falling oil prices have had an effect on recruitment even in traditionally "safe" subject areas; some university careers offices have reported problems among civil and mechanical engineers, for example. The number of companies that recruit on the "milk-round" of universities has decreased this year (although the decrease

Per cent home first graduates unemployed, shown according to discipline

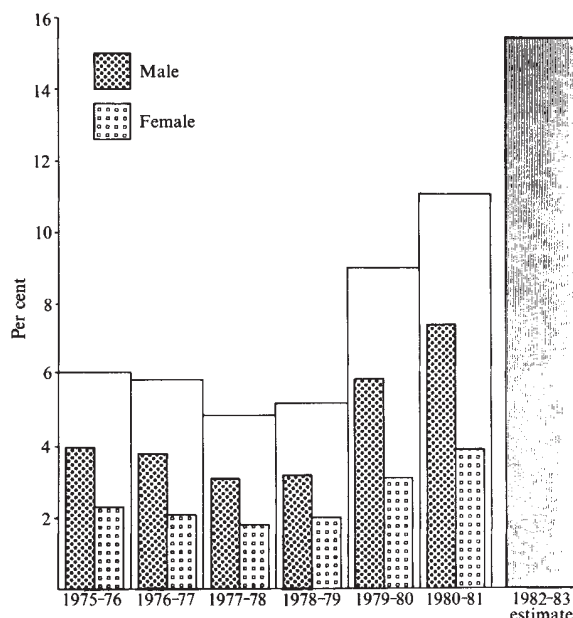


- A Architecture and other professional and vocational studies
- B Agriculture, forestry and veterinary science
- C Medicine, dentistry and health
- D Education

is not large), and there are other indications that the number of advertised vacancies for graduates has fallen over the past two years. The sudden growth in the number of sponsorship schemes since 1979 (see accompanying figure) accounts for a part of this decrease, and it seems that the number of vacancies has probably reached a plateau. It is unlikely to increase significantly for at least 18 months, even if there were to be a major reflation of the economy next year. **Tim Beardsley**

Home first degree graduates believed unemployed at 31 December

All values are expressed as percentages of the total number of graduates of known destinations. About 10 per cent of graduates did not reply to survey questionnaires. The 1982 figure is an informed estimate.



CORRESPONDENCE

Orang is man

SIR — I have long resisted the temptation to point out the erroneous practice of many Western editors and authors of abbreviating 'orang' for 'orang-utan'. Reference to any Malay or Indonesian dictionary will confirm that 'orang' means 'man' and 'orang-utan' means 'orang-utan'. Therefore, when Lowenstein, Molleson and Washburn reported that they have results to prove that 'both the Piltown jaw and the canine tooth are those of an *orang-utan*' and you have their letter headlined "Piltown jaw confirmed as orang" (*Nature* 23 September, p.294), I hope you are guilty only of misleading your readers with an inappropriate abbreviation and not of misunderstanding the key issue of the debate!

J. FLEURIS

Quebec, Canada

"IT'S ALL EXPLAINED IN THE
THOUGHTS OF CHAIRWOMAN MAO."



Chinese puzzles

SIR — In *Nature* of 16 September (p.200) J.A. Irving wrote to point out that Chinese surnames precede given names. This is no longer a problem in English translation because given names are now hyphenated and the second one is not capitalized. For foreigners, however, the gender of nouns and pronouns remains confusing. For instance, "Mr" Qi, cited by Irving, is actually Mrs Qi.

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War prevention

SIR — Jeffrey Segall (*Nature* 4 November, p.10) attributes to me (*Nature* 7 October, p.482) approval of the Medical Association for the Prevention of War (MAWP) proposals for a "Comprehensive ban on all methods and means of warfare specifically designed to kill or injure by inducing human disease". He fails to see two separate strands in my comments on MAWP.

Firstly, I consider the existing international prohibitions and other restrictions on warfare as among the highest achievements of man.

We should all hope for a wider and more effective extension where appropriate of their type of international agreement. However, without diminishing their significance, it should be recognized that their success lies in that they involve noncentral areas of military concern.

Secondly, we should not restrict our attention only to the salient and direct causes of suffering involved in war. The concern with the methods and means of warfare should not distort our perception to ignore the fact that wars are initiated in the pursuit of military and political goals. And that prevention and control of these goals are as significant, if not more so, as that of their means for stopping war and the suffering within them.

Unfortunately not all desirable things are compatible. The inhibition of the initiation of wars depends largely on deterrence which requires armaments. The removal of the world's dependence on deterrence is likely to be achieved not by disarmament but by either an ascendancy of one of the superpowers to be effectively the only world power, or the creation of internal political structures of inhibition within military superpowers. But the latter and most desirable change would require the unlikely development of political pluralism, autonomous judiciary and intellectually open societies in the at present totalitarian powers of the Soviet Union and China.

J.R. SKOYLES

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Malaria resurgence

SIR — Chapin and Wasserstrom¹ stated that resurgence of malaria in India was the result of the introduction of high yielding varieties (HYV) of rice and cotton, and we disputed the basic tenets of this hypothesis². The main thrust of their argument now, however, seems to be that resurgence was the result of the use of insecticides in agriculture³. We would like to add a few further comments.

(1) Widespread resistance to common pesticides in anophelines developed during early and mid-1960s. *Anopheles culicifacies* was found resistant to DDT in 271 units in 14 states and to BHC in 24 units in 4 states. Similarly *Anopheles stephensi* was found resistant to DDT in 34 units in 5 states and to BHC in 6 units in 3 states⁴.

(2) *A. stephensi* type form, the vector of urban malaria in India, breeds in cisterns, overhead tanks, garden tubs, fountain basins, disused containers, receptacles used for storing water, and other water accumulations in cities and does not breed in agricultural fields. Resurgence of malaria was observed in cities of India from southern, central and north India⁵.

(3) Although accurate quantities of DDT used in agriculture (1960–72) are not known, the available figures need not be extrapolated as they are about correct with minor variations.

(4) It is usual for genetic mutations for insecticide resistance to be less "fit", and reversion towards susceptibility generally

occurs in the absence of insecticidal pressure. This was observed in *A. culicifacies* after withdrawal of spraying or change of insecticides in Maharashtra state in 12 units (28 villages)⁴.

(5) Irrigation schemes contributed to the proliferation of the existing mosquito population which had become resistant in large parts of the country. It is incorrect to assume that the process of selection started *de novo* in the newly irrigated fields.

(6) In certain areas, a certain degree of contribution of the use of insecticide in agriculture in accelerating the pace of resistance in vectors of public health importance is understandable, and examples can be found to support this view. But it is difficult to believe that the massive quantities of insecticides used in public health and directed towards the malaria vectors were not the primary cause of widespread insecticide resistance.

The doubts raised by Chapin and Wasserstrom about the value of high-yielding crop varieties are a serious matter for a developing world striving hard to increase food production by introducing such varieties. The singling out of these crops as the main culprits for the resurgence of malaria without any authentic data is unjustified, and has been rightly condemned by Bruce-Chwatt as a "garbled account"⁶.

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2. Sharma, V.P. & Mehrotra, K.N. *Nature* **298**, 210 (1982).
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Muscling in

SIR — It is very misleading for muscle biophysicists to continue to refer to the anterior byssal retractor muscle (ABRM) of *Mytilus* as an example of unstriated (= smooth) muscle (*Nature* **299**, 308; 1980). For almost 30 years, since the ABRM was first examined by electron microscopy, it has been realized that this muscle in fact enjoys a very regular, striated, ultrastructure. There is a highly orderly arrangement of thin and thick filaments, although they are arranged obliquely rather than transversely, in the relaxed muscle. The correct designation for this type of muscle is *obliquely-striated*.

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NEWS AND VIEWS

More speculation about oncogenes

Last week's reports of the surprisingly simple difference between a normal human gene and its equivalent in a human cancer cell is not merely important but provoking. What will happen next?

What follows is pure speculation. Those of an austere temperament should turn the page. Others, especially armchair molecular biologists, may welcome an opportunity to compare their private musings with the public hostages to fortune below. But in reality, nobody needs to be ashamed, for the discoveries described last week lead directly to a paradox.

The discoveries^{1,2} are concerned with the difference between a normal human gene responsible for the production of a protein called p21 and the corresponding gene in cells derived from a bladder carcinoma; the difference consists of the replacement of one nucleotide by another precisely thirty-five units from the point in the functional gene at which the transcription of DNA into RNA begins. So the presence of the abnormal gene is a sign that cells derived from the bladder cancer have the properties of cancer cells, but not of course a proof that this simple mutation caused the malignancy in the first place. (This does not diminish the importance of the discovery, whose immediate interest is that it points directly to the protein p21 as one whose unknown function must play a crucial role in the biochemical functioning of both normal and malignant cells.) But it is also known that the mouse cells used as touchstones of carcinogenesis, peculiar though they may be, are also made cancerous ("transformed") when they are dosed with the normal gene for p21 provided that it is suitably equipped with the nucleotide sequences known to be necessary for viral replication in a vertebrate cell. The paradox is merely the question why the production of a small amount of an abnormal protein should have physiological consequences very similar to those of the production of an abnormally large amount of the normal protein.

Paradox

A paradox is a conflict between the conclusions of two apparently logical arguments. Paradoxes are usually resolved by the discovery that one argument or the other contains a hidden flaw, often of a semantic character. Can the Weinberg-Barbacid paradox be as easily resolved? That could be most simply accomplished if a little of the abnormal version of p21 and a lot of the normal protein were not different from each other but identical, at least in respect of the cancerous properties of the transformed cells. But how could that be? Both groups reporting last week point out that the effect of the genetic mutation they have found is that the simple amino acid glycine at position twelve in normal p21 is replaced by valine in the abnormal version, from which they infer that the altered protein molecules are less likely than normally occurring p21 to begin (from the end carrying a free amino-group) with a tidy coil of roughly helical structure.

This comparison cannot however be exact. Even the tidiest helical protein structure is not helical forever. It must be supposed that from time to time the helical ends of normal p21 will be unwound, or that there will be some temperature-dependent proportion of normal p21 molecules that have the configuration now inferred for the abnormal variety. So is it possible that the similarity of the effects of small amounts of abnormal protein and large amounts of the normal variety, at least in respect of their cancerous properties, arises because large amounts of the normal protein provide small (but "sufficiently large") amounts of the abnormal configuration? That would be a semantic resolution of the paradox.

This speculation (it is not an argument) then raises the intriguing possibility that normal p21 in normal cells has two

distinct functions, one involving the more common tidy helical structure of the first score or so of amino acids, the other requiring a structure similar to that inferred for the abnormal protein. So far, so good, but even speculation now abruptly stops. The normal function of normal p21 is entirely obscure, so that it is profitless to go guessing at the abnormal function. Fortunately, real molecular biologists will not be at a loss. Indeed, one of the obvious lines of enquiry now suggested is to use the techniques of genetic manipulation to right the changes on the first dozen or so amino acids of p21 to discover which parts of the structure are essential for the transforming activity of the abnormal gene. Telling how that part of the molecule functions will require more luck.

Evolution

This line of speculation leads to another. If the presence of an abnormal gene for p21 in a human bladder cell can have such profound effects, and if the frequency of random mutations is as great as often supposed, by what means in the evolution of vertebrates has it been arranged that these lethal mutations are selectively suppressed? The question is more taxing than that of understanding the conservation of other genes that have survived long stretches of evolution more or less unchanged, for then it is at least possible to suppose that a cell inheriting an unwanted mutation will simply die unnoticed. But if taken at its face value, a mutation at position 35 of the p21 gene (or presumably at the preceeding one as well) will transform the cell which carries it into a form that grows successfully, and which proliferates. So it is natural to postulate some biochemical mechanism for removing these lethal mutations before than can do much damage.

This, of course, is not a novel speculation. Armchair oncologists were onto it while molecular biology was in its infancy. But two plausible conclusions follow. First, if the difference between a normal and a cancer cell is as delicate as now appears, the case for an active molecular mechanism for removing unwanted mutations is strengthened (which is not to suggest that it is within sight of being understood). And second, given such a mechanism, the occurrence of the abnormal gene in cells taken from a human bladder cancer is much more likely to be the repository of the mechanism of cancer than its first cause. That is just as well, for it would otherwise be hard to account for the long induction period associated with most forms of cancer, as everybody has pointed out.

The snag, with speculation in this or any other fields, is that it cannot solve puzzles but only replace one puzzle by another. The discoveries reported last week by the Weinberg and Barbacid groups, are themselves based on a new technology patiently developed over the past decade, and on a mass of painfully acquired and still poorly-assimilated information about the behaviour of retroviruses (which carry genetic information as RNA, but which can integrate as DNA among the genes of the cells that they infect). They are sensational because they are so unexpected and because they have been made at the bench, not in the armchair. That is also how the next surprises will arise in this now exciting chase. They will not be long delayed.

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Natural killers to rescue immune surveillance?

from Barry R. Bloom

DESPITE the myriad difficulties in finding definitive experimental verification, the idea of immunological surveillance holds a unique appeal for cellular immunologists. The existence of a surveillance mechanism for the continuous and systematic rejection of altered body cells was first inferred when it was found that specific cytotoxic T lymphocytes (CTLs)¹ could lyse allogeneic (non-self) cells and syngeneic (self) virus-infected or tumour cells. Soon apparent, however, was the disconcerting fact that athymic nude mice, which lack this mechanism, had no greater incidence of primary spontaneous, virally or chemically induced neoplasia than normal mice. It is in this context that the discovery of natural killer (NK) cells — present in both athymic and normal mice — renewed hopes that immunological surveillance might be a real phenomenon.

Natural killer cells represent an apparently heterogeneous population of cells with the capability of spontaneously lysing a variety of transformed cells, virus-infected cells and embryonal cells *in vitro*, without evident prior exposure to specific antigen *in vivo*. The revelation that NK activity is regulated by interferons, and also by interleukin (IL)-2, markedly heightened interest in these cells, if not their credibility.

Despite the extraordinary amount of information generated about NK cells in a very short period of time, recently reviewed at an excellent workshop* on natural killing, the most fundamental questions — the provenance and lineage of NK cells, their mode of recognition and their function *in vivo* — remain problematic.

The dogma implicit in much of cellular immunology, 'By their markers, shall they be known', is currently being confounded by NK cells. A large number of surface determinants have been identified on NK cells and have helped to establish their heterogeneity and define subsets, but most, if not all, of the markers are found on other cell types as well. In the mouse, NK cells, although lacking the Lyt 1 and Lyt 2 markers of helper and suppressor/cytotoxic T subsets, do share Thy 1, Qa4 and -5, Ly5 and asialo-GM₁ with T cells. Human NK cells express the OKT3, OKT6, OKT11 and to a lesser extent the OKT4 and OKT8 markers associated with immature and differentiated T cells. However, they also express the OKM1 marker found on monocytes and granulocytes. Murine NK cells express, in addition to Fc receptors for IgG, high-affinity IgE receptors, similar to mast cells and basophils. Worse yet, in short-term culture, the expression of markers can vary qualitatively from day to

day¹. Even the most useful and specific monoclonal antibodies (anti-NK1.1 in the mouse and HNK-1 [Leu/] in man) react with a greater proportion of cells than can be accounted for by NK activity. The recent observation (Stutman, Sloan-Kettering Institute) that one NK cell subset, termed NC cells, known not to be affected by interferon, is regulated by IL-3 which is also a mast cell growth factor, raises the possibility that specific growth factors may ultimately be helpful in resolving questions of NK cell lineage.

One of the controversies that has smouldered over the years is the relationship between NK cells and K cells, the latter being defined as cells possessing Fc receptors for IgG that have the ability to lyse antibody-coated target cells *in vitro*. These cells express almost all known NK markers yet clearly have a different mode of recognition and killing of target cells. Using the single-cell cytotoxicity assay, individual cells were shown to kill both NK-susceptible and antibody-coated targets, demonstrating that the same cell could carry out both activities². Only in the germ-free piglet does that dichotomy remain (Kim, Sloan-Kettering Institute).

The selectivity or specificity of NK cells for certain target cells remains enigmatic. Many cloned NK lines have the ability to kill a wide variety of targets, including some not recognized by primary NK cells, yet some human clones have quite remarkable selectivity for only certain targets. It is unclear why some tumour or virus-infected cells are not lysed, and why some embryonal and normal cells are either killed or are capable of blocking killing of appropriate NK targets. Wigzell (Karolinska Institute) reported the intriguing finding of NK-like activity in two of six rat tumour-specific CTL lines. The cells had classical MHC (major histocompatibility complex)-restricted killing of the specific syngeneic chemically induced tumour target, but in addition could lyse xenogeneic NK targets (targets from a different species). An anti-idiotypic antibody was obtained which blocked the MHC-restricted tumour-specific killing, but did not affect the killing of the NK targets. This clearly indicated that recognition of the tumour-specific antigen and NK target structures are independent, and that receptors for NK targets are distinct from those for MHC-restricted specific antigens. It will be of interest to know whether exposure of these cells to the NK targets will clonally expand them for tumour-specific killing. Recently, human NK-like clones derived from mixed lymphocyte culture were found to have clonally distributed receptors distinct from MHC recognition³. These and most other experiments on NK cell recognition raise

the question of whether there is a clonally distributed diversity of NK receptors for various target cells, or whether there is a class of surface structures common to some tumour cells, virus-infected cells and embryonal cells recognized by most NK cells.

If the mode of NK cell recognition remains mysterious, the mechanism by which they kill their targets is being solved, to the extent that they have become the paradigm for understanding cell-mediated cytotoxic mechanisms. Human and rat, but to a much lesser degree mouse, NK cells appear as large granular lymphocytes (LGLs), morphologically quite distinct from other lymphoid cells. A wide variety of studies ranging from microcinematography to the use of selective metabolic inhibitors indicate that lysis is mediated by vesicular secretion. Thus drugs that block vesicular secretion in other cell types, for example, mast cells, inhibit NK cell killing^{4,5}; agents that degranulate, such as Sr²⁺, both deplete the granules from LGLs and inhibit killing⁶. The stimulus-secretion model, initially suggested for NK cells by Roder, would hold that the critical requirements for killing are (1) binding to target cells, (2) movement of the uropod containing the granules into apposition to the target⁷, (3) Ca²⁺-dependent degranulation or release of an NK cytotoxic molecule, (4) binding of the cytotoxic factor to the target and (5) lysis of the target by the released factor. An NK cytotoxic factor has been found to be released from mouse NK cells and human LGLs on interaction with NK-susceptible targets^{8,9}, which has the ability to lyse NK-susceptible as well as some resistant targets. Target cells can be resistant to NK killing if they either fail to bind NK cells, or fail to bind or succumb to the cytotoxic factor. Because several laboratories failed to find production of oxygen radicals or inhibition of NK killing with radical scavengers, a proposed role for oxygen metabolites in mediating NK killing¹⁰ has been met with some scepticism. If there are independent receptors for binding of NK cells and an NK cytotoxic factor, these mechanistic studies can explain some of the apparent selectivity for killing various target cells. From these studies, one hopes it will also be possible to define the nature of the NK cell defects found in several disease states.

To achieve credibility NK cells must be shown to perform some important functions *in vivo*. Many years ago, the late Gustavo Cudkovicz and collaborators¹¹ found a genetic association between NK activity and the acute rejection of allo-

*'Workshop on Natural Killing', held at Quail Roost, North Carolina on 31 August-3 September 1982, was organized by H.S. Koren and R.B. Heiberger.

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geneic bone marrow grafts. The antigen that was recognized in this system was found to be closely linked to the *H-2D* locus. Studies on this haematopoietic histocompatibility locus gave rise to the notion that NK cells may be important not only for acute rejection of bone marrow and other grafts, but also for maintaining regulation or surveillance of the bone marrow itself.

Genetically NK cell-deficient *beige* mice as well as mice selectively depleted of NK cell function by treatment with antibodies to NK1.1 antigen, asialo-GM1, or mouse interferon are found to have increased metastases of a variety of transplanted tumour cells, suggesting that NK cells have a role in restricting metastases. It has been very difficult to show, however, even in *beige* mice, that NK cells have a significant role in restricting spontaneous viral or chemical tumorigenesis. One difficulty in these studies is that almost all chemical and viral carcinogens profoundly depress NK cell function, often for very long periods of time. In a recent issue of *Nature* (4 November, p.31), Warner and Dennert provide compelling evidence that NK cells can mediate three major surveillance functions *in vivo* — rejection of bone marrow grafts, restriction of tumour metastases and inhibition of primary tumorigenesis. Dennert was the first to report continuous cloned cell lines with NK-like activity, and the ability of cloned NK lines to mediate these three surveillance

functions *in vivo* suggests that the power of cloned cell lines will be no less great in providing a unique tool for studying mechanisms and functions of NK cells than it has been in the case of B and T cells. While questions remain whether the effects are mediated directly or indirectly by the cloned cell lines, and what the appropriate growth factors are that maintain the cells in an active state *in vivo*, these experiments provide direct evidence that NK cells can exert a profound regulatory role in acute graft rejection and in restriction of neoplasia and metastases. They provide an important model for exploring the role of NK cells in controlling viral, bacterial and parasitic infections *in vivo*. They will also be cited to justify the possibility of ultimately (in immunology, that means within two years) intervening therapeutically in human disease using purified LGLs or cloned NK cells.

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with supporting temperature sensor next identified hot water⁶ (admittedly only 0.1°C hotter than surrounding ambient seawater) associated with volcanic activity at the crest of the back-arc basin 'rift valley.' Metal deposits usually associated with massive generation of sulphide ore at sea-floor hot springs were also dredged. Now, Yoshio Horibe of the Ocean Research Institute, University of Tokyo, and Kyung-Ryul Kim and Harmon Craig of the Scripps Institution of Oceanography have uncovered conclusive evidence for the existence of hot vents in the Mariana Trough⁷.

Hot vents carry rare gases from the magma below the sea floor to the ocean column. Thus if samples from the water column above suspected vent sites³ are analysed, helium, hydrogen and methane anomalies can be found. This technique successfully searched out hot springs at 20°S on the East Pacific Rise.

Aboard the research vessel *Hakuho-maru* of the University of Tokyo, samples *m* from the back-arc basin spreading centre and from the waters above the two-watt power source were analysed for methane in February 1982. Methane is a clear product of hydrothermal vents in the eastern Pacific, but is consumed so quickly by bacteria floating in the ocean that a methane anomaly requires an active vent to spew hot hydrothermal waters into the ocean.

First, the scientists went to the two-watt area but no methane anomaly was found — the heat must be a remnant of a now-dead vent. Then at the back-arc basin spreading centre, the largest methane anomaly ever found in the oceans was recorded (see the figure). Later shore-based analysis verified this finding by showing a staggering 26 per cent increase in primordial ³He. Hot vents must be active at the crest of the Mariana Trough back-arc basin.

Why the excitement? Vents are likely to exist all up and down the East Pacific Rise crest. Consider the biology. Will any living creatures be found? If so, will they be similar to those in the eastern Pacific: the large eastern Pacific clams are a form of mollusc indigenous to the northeastern coast of North America. Will the Mariana Trough have 'Japanese' clams? Will the spectacularly coloured tube-worms be

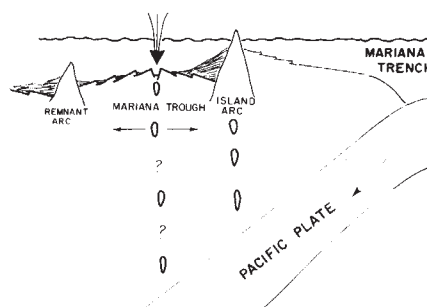
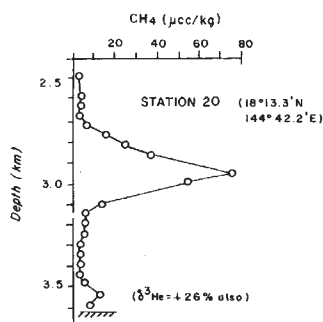
Call Alvin for hot science

from Roger N. Anderson

RECENT discoveries of deep-ocean hydrothermal vents in the Mariana Trough back-arc basin have stirred new excitement within the geological and biological communities. In the past few years, hot springs billowing forth metal-laden waters at temperatures up to 350°C have been found all along the East Pacific Rise crest from 21°N to 12°N and 20°S. Fantastic and unique biological communities live near these hot springs, ingesting sulphur-eating bacteria. But to date, all these organisms and associated 'black-smokers' have been found in the eastern Pacific close to a mid-ocean ridge. Now a new hot-spring vent area has been found — not only is it in the western Pacific but it is also above a subduction zone, where the Pacific Plate is being forced under the Philippine Plate. Both the biological and geological settings are in distinct contrast to the eastern Pacific hot vents. Scientists are clamouring to document similarities and

differences between the mid-ocean ridge hot vents and the back-arc basin vents.

But first the evidence. As with the previous hot spring discoveries^{3,4}, oceanic heat flow measurements were the first to suggest hot-vent activity in the Mariana Trough⁵. Very hot patches of sea floor emitting more than two watts of geothermal power per metre (50 times normal) and bizarre temperature-depth recordings were strong hints of hot spring activity. A deeply towed acoustic imager



Schematic drawing of Mariana Trough back-arc spreading basin with methane anomaly measured in water column above rift valley (arrowed) by Horilu *et al.*⁷

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present? Jack Corliss of Oregon State University has even postulated that life on Earth originated at 'black-smokers'!

Now to the geology: the subduction zone clearly supplies volatiles (for example, water to the volcanoes of the island arcs). Does the addition of this volatile source to the back-arc basin spreading centre change the chemical composition of the hot springs? And if so, does it change the metallogenesis occurring at spreading centres versus that at back-arc basins? These hot vents produce a significant quantity (about 20 per cent) of the mined metals in the world. Here is a chance to see the formation of metals in action today.

What next? Now it is time for the diving: submarine *Alvin* to sample these Mariana Trough hot vents. Plans are being formulated for a diving season in winter-

spring 1984-1985 in the western Pacific. Those of you who might have unique science to offer to the exploration of the Mariana Trough hot vents should contact the *Alvin* Review Committee, University National Ocean Laboratories (UNOLS) Office, Department of Oceanography, University of Washington, Seattle. □

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Gene conversion between repeated genes

from Thomas Petes and Gerald R. Fink

IN the now 'classical' studies of gene conversion, the conversion of one gene to another occurs only among allelic genes present in single copies on homologous chromosomes. Recent work indicates, however, that among repeated genes, conversion can occur even when the genes are located on non-homologous chromosomes. In this article, we will discuss both examples of gene conversion and also the recent suggestion from Egel¹ and Baltimore² that gene conversion events occur not only in fungi but also in the cells of higher mammals. We consider that gene conversion in fungi and recombination events in mammalian cells might well be different.

Fungi are particularly suited to the study of gene conversion because all the progeny from a single fungal meiosis are sequestered in a structure called the ascus. For a heterozygous pair of alleles *A* (wild type) and *a* (mutant), the expected pattern of meiotic segregation is 2*A* segregants and 2*a* segregants. Gene conversion is detected by deviations of the type 3*A*:1*a* or 1*A*:3*a*. In tetrads with 1*A*:3*a* segregation, Roman³ showed that all three mutant alleles were identical. Thus gene conversion represents non-reciprocal precise transfer of information between two genes, either from *A* to *a* or from *a* to *A*. Extensive genetic analysis of this process has resulted in several other generalizations. For all loci, conversion can occur either from mutant to wild type or wild type to mutant. In *Saccharomyces cerevisiae*, conversion is often equally frequent in both directions, although significant deviations in one direction have been seen⁴. Meiotic gene conversion in *S. cerevisiae* occurs but at a

frequency of about one per cent per locus. Mitotic gene conversion also occurs but at a much lower frequency (10^{-4} - 10^{-6}). Deletions and insertions are converted at frequencies comparable with those found for point mutations⁵. For all organisms where genetic analysis has been possible conversion is non-randomly associated with reciprocal recombination⁶.

Holliday⁷ suggested that gene conversion is the result of heteroduplex formation between allelic genes, followed by mismatch repair. Mismatch repair involves the excision of one strand of the heteroduplex followed by resynthesis using the unexcised complementary strand as a template. This mechanism allows the faithful transfer of information from one gene to another. Holliday and others⁸ have proposed that heteroduplex formation, which creates a potential site for gene conversion, is an obligatory intermediate in the process of reciprocal recombination.

Recombination events between single-copy genes are restricted to events between equivalent sites on homologous chromosomes, but repeated genes on nonhomologous chromosomes should also be capable of recombinational interactions. Using recombinant DNA procedures and the yeast transformation techniques, Scherer and Davis⁹ constructed haploid *S. cerevisiae* strains with two copies of the *his3* gene (each copy

containing a different small deletion); the genes were located on nonhomologous chromosomes. Since the wild-type *his3* gene was formed without concomitant formation of the double mutant, they concluded that *His*⁺ recombinants were generated by gene conversion. Similar procedures were used to show that mitotic gene conversion occurs between repeated genes on the same chromosome¹⁰. Gene conversion has also been seen between naturally occurring repeated yeast genes. Ernst *et al.*¹¹ showed that some revertants of iso-1-cytochrome *c* mutations were the result of gene conversion between the partially homologous iso-1- and iso-2-cytochrome *c* genes. All these examples of gene conversion between repeated genes in *S. cerevisiae* are mitotic events but meiotic intrachromosomal gene conversion has also been demonstrated¹¹.

Meiotic gene conversion between repeated tRNA genes in the fission yeast *Schizosaccharomyces pombe* is reported by Munz and his colleagues in this issue of *Nature* (p.225). They show that an inactive UGA-reading serine tRNA suppressor gene (the suppressing activity lost because of a secondary mutation in the tRNA gene) can be re-activated by a recombination event. Assuming that all the secondary inactivating mutations used in the study do not have an extremely high rate of reversion, Munz *et al.* suggest that the inactivating mutations are removed by conversational interactions with other serine tRNA genes in the genome — one of these is on the same chromosome as the serine suppressor tRNA, the other is on a different chromosome. In support of these conclusions, they show by Southern blot analysis and DNA sequencing that serine tRNA genes can be altered in a single genetic event at multiple positions within the gene. Such alterations are much more easily explained as gene conversion events than as mutations. Remarkably, there is less than 200 base pairs of homology between the interacting tRNA genes which undergo this conversion event.

Gene conversion events between repeated genes, such as those described above, are likely to have two important genetic consequences. First, continued cycles of gene conversion within a family of repeated genes could lead to the maintenance of sequence homogeneity within that family¹⁴. Second, gene conversion can produce sequence diversity since conversion between repeats that differ at more than one position can form new combinations of sequences^{1,2,9-12}. The net effect of conversion is likely to be a complicated function of the rate of conversion relative to mutation, the location of the interacting repeats, the length of the repeats and other factors.

Since gene conversion between repeated genes is likely to have important genetic consequences, it is obviously of interest to determine whether this type of conversion occurs in higher cells. Slightom *et al.*¹⁵

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found that the duplicated human γ^A and γ^G genes within a single chromosome were more similar than two allelic γ^A genes. They proposed that intrachromosomal gene conversion was the most economical explanation of the data. Similar observations have also been made recently for several other classes of repeated genes in higher organisms^{1,2,16}.

We believe that gene conversion may explain some recombination events between repeated genes in mammalian cells, but the process is yet to be demonstrated experimentally and may be different from that found in fungi. The observations in higher eukaryotes can be explained by unequal double cross-overs as well as by conversion. So far, no one has developed a system in higher organisms that allows examination of both participants in the recombination event before and after the postulated conversion. In considering gene conversion as a mechanism that maintains homogeneity among repeated genes¹⁴, it is important to remember that conversion in fungal systems is often associated with reciprocal recombination⁶. It is not clear, therefore, how frequent cycles of gene conversion would be dissociated from the production of chromosomal deletions, inversions and translocations. This problem should be particularly acute in mammalian genomes where repeated sequences constitute a large fraction of the DNA. It is possible that in mammalian cells gene conversion is not obligatorily associated with reciprocal recombination. Even in fungi there is a class of mitotic gene conversions that are not associated with reciprocal recombination^{10,17,18}. Over the next few years, it is likely that a combination of recombinant DNA techniques and DNA-mediated transformation will help to determine whether the recombination events observed in mammalian cells have all the properties of gene conversion events in fungi.

The origin of tektites — settled at last?

from Peter J. Smith

TEKTITES, those small glassy objects limited to a few well-defined regions of the Earth's surface, have haunted geologists for more than a century. Like country house ghosts, they have never been part of the investigative mainstream but have given rise to a variety of weird and wonderful explanations that attract the occasional attention of the wider community particularly when it has seemed that a new technique might throw some light on their nature and origin. Unlike country house ghosts, on the other hand, they are undoubtedly real, having been regarded as fine ornamental material since at least the time of Cro-Magnon man, about 31,000 years ago.

It was perhaps unfortunate that early scientific attention should have been directed towards Czechoslovakian tektites (moldavites) from a region of ancient European glassmaking. The result, during the nineteenth and early twentieth centuries, was a long time-wasting debate over whether tektites are real or artificial, productive only in the sense that the right conclusion was reached in the end. By then, however, it had already become clear that if tektites were natural they were not to be easily explained, for there was no obvious source, or 'mother lode'. The way was thus open to a wide range of possible explanations from the down-to-earth (literally and figuratively) to the bizarre.

Terrestrial volcanism was the obvious first choice; but it soon became evident that there were significant chemical differences between tektites and obsidian, the more common glassy material of undisputedly volcanic origin. Besides, candidate volcanoes close to the tektite zones, or 'strewnfields', were hard to find. To avoid some of these difficulties, Dunn (*Victoria, Geol. Surv. Bull.* 27, 3; 1912) proposed an elaborate derivation of tektites from obsidian, involving lightning strikes in a very dusty atmosphere packed with hot gases, but it was not sufficient to overcome the objection that tektite compositions were far removed from those of terrestrial volcanics generally. During subsequent decades, tektites were to be attributed to, among other things, the heating of stony meteorites, the collision between a meteorite and a natural Earth satellite, the drying-out of siliceous gels acted upon by humic acid, and the fall of antimatter, although neither these nor many other hypotheses stood the test of time, if they ever stood at all.

What we are left with in 1982 are just two contending hypotheses, both of which involve extraterrestrial bodies. According to one, tektites are volcanic products ejected from the Moon; and according to

the other, they are fragments ejected from the Earth itself by cometary or meteoritic impact. As work on returned lunar samples has proceeded, a lunar volcanic origin of tektites has come to seem less and less likely, although there are those, most notably O'Keefe (*Geochim. cosmochim. Acta* 44, 2151; 1981), who would strongly dispute that view. The alternative explanation, by contrast, has risen in popularity, bolstered no doubt by a natural desire to avoid exotic solutions wherever possible. The hot money these days is on the idea that tektites result from the impact melting of terrestrial rocks, especially sediments; and the odds in favour of that must surely have risen with the publication by Shaw and Wasserburg (*Earth planet. Sci. Lett.* 60, 155; 1982) of a detailed study of the world's tektites using Sm-Nd and Rb-Sr systematics.

There are four tektite strewnfields, within each of which the tektites have the same K-Ar and fission track ages. Thus the North American, European, Ivory Coast and Australasian tektites are about 35, 14, 1.3 and 0.7 million years old respectively. These ages represent the times at which the tektites were last molten; they say little about the types of rock from which the tektites were formed, how they formed, or whence they came. Fortunately, however, it is now possible to determine rather more, both comparatively and absolutely, about the provenance and evolution of rocks from the behaviour of the Sm-Nd and Rb-Sr decay systems.

As for the comparative, one way of expressing isotopic ratios is to use the ϵ notation. Thus ϵ^{Nd} (0) is the measured deviation (in parts in 10^4) of the $^{143}\text{Nd}/^{144}\text{Nd}$ ratio from the present-day chondritic value of 0.511847; and ϵ^{Sr} (0) is the deviation (also in parts in 10^4) of the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio from a model undifferentiated mantle reservoir value of 0.7045. The point about ϵ values is simply that they are different for different rock types. Shaw and Wasserburg have measured ϵ values for geographically and geochemically representative samples from all four tektite strewnfields and find that ϵ^{Nd} (0) is always negative and ϵ^{Sr} (0) is always positive and large. These values are characteristic of old terrestrial continental crust. They exclude continental material which has been derived from the mantle very recently, for that has ϵ values close to zero; and they exclude oceanic crust, because that has positive ϵ^{Nd} (0) values and negative ϵ^{Sr} (0) values. The isotopic data thus match the chemical data, for, as many

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workers have pointed out since at least 1917, the chemistry of tektites resembles nothing so much as continental sediments, especially sandstones. There are no known lunar rocks having major and trace element compositions similar to those of tektites, and there are no known meteorites with these compositions.

The second important discovery made by Shaw and Wasserburg is that within each tektite group $\epsilon^{Nd}(0)$ is uniform whereas $\epsilon^{Sr}(0)$ is highly variable. This reflects what earth scientists already suppose about the different types of information provided by the Sm-Nd and Rb-Sr systems. Assuming that concentrations are high enough to measure, any radioactive decay system will give an age for the rock concerned, but the term 'age' needs careful interpretation. The major fractionation of Sm and Nd appears to occur during the partial melting of mantle material to produce crust, and the Sm-Nd system is relatively undisturbed by such processes as weathering and sedimentation. Moreover, Sm and Nd are refractory and thus not readily fractionated by differential volatilization on impact melting. The Sm-Nd age of a tektite should therefore represent the time of formation of the crustal material from which the tektite was ultimately derived, and uniformity of $\epsilon^{Nd}(0)$ within a single tektite group inspires confidence that the Sm-Nd system is dating precisely that event. The Rb-Sr system, by contrast, can be severely disturbed by sedimentation, volatilization, weathering and diagenesis. It is only to be expected, therefore, that a tektite group will provide a range of $\epsilon^{Sr}(0)$ values and Rb-Sr ages reflecting the varied history of the tektite source rocks and descending to the last major parent-daughter fractionation event. In short, if nature is behaving itself, all tektites within a group should have a common Sm-Nd age, a range of younger Rb-Sr ages and, of course, a common and even younger K-Ar age representing the time at which the tektite themselves formed.

And thus it turns out. The Sm-Nd ages obtained by Shaw and Wasserburg for the North American, European, Ivory Coast and Australasian tektites are, respectively, about 650, 900, 1,900 and 1,150 million years. In other words, all the ages are consistent with the tektites' having been formed from Precambrian crust. The Rb-Sr data then indicate what happened between the formation of that crust and its conversion to tektite. The European tektites, for example, were derived from sediments laid down only about 20 million years ago. Interestingly, sediments in the nearby Ries Crater have ϵ values and isotopic compositions similar to those of the tektites themselves. Moreover, the age of the oldest basement in the crater area agrees well with the Sm-Nd age of the tektites. The new data thus support an earlier suggestion that the Ries Crater was formed by the impacting body that also



WHILE watching the grand display of aurora on Friday night from our roof, at about 6h. 7m., my wife and I saw a strange gleam of light rising above a bank of cloud on the eastern horizon, nearly vertically below the Pleiades, like the gleam of another moon rising in a haze. It grew out slowly, as we watched it, into a strong beam of white light slanting towards the south, and we stood in wonderment as it lengthened out making straight towards the moon. Presently its tail was dis-engaged from the cloud, and it stole through the sky like a long luminous nebulous "cigar ship" exactly across the moon, and away down into the west, sinking as slowly as it had risen. You will probably receive many accounts of this strange apparition. It will be interesting to know the position relative to the moon in which it was seen by different observers. Was it clear of the earth's atmosphere or not?

HUBERT AIRY

Woodbridge, November 19, 1882.

gave rise to the tektites.

The Ivory Coast strewnfield has also been associated with an impact structure, namely, the Bosumtwi Crater. The rocks around this crater have ages comparable with the Sm-Nd age of the tektites. The Rb-Sr systematics indicate that the tektites were formed from Precambrian sediments about 950 million years old, although the sediments have not been identified yet. The North American tektites, by contrast, have no known source crater. Moreover, the fact that the material that went to form the tektites was derived from the mantle as recently as the late Precambrian rules out most of the North American Precambrian shield, and the sediments derived therefrom, as a source region. Similar age criteria refute the recent suggestion from Dietz (*Meteoritics* 12, 145; 1977) that the Popigai astrobleme of Siberia is the source of the North American strewnfield. The only known continental material of the required age is the late Precambrian crust constituting the east-southeast margin of the Appalachian orogenic belt, although no suitable crater has been identified within it.

Little more can be said about the origin of the North American tektites, except that the immediate source material was sedimentary, for the Rb-Sr proved unsuitable for the determination of an age of sedimentation. Not the least interesting conclusion to be drawn from the work of Shaw and Wasserburg, however, is that an oceanic impact for the origin of tektites is not ruled out by the restriction that tektite source material cannot be oceanic crust. Although the effects of the impact at the Ries Crater extend to depths of about 2 km, the tektites supposedly produced by the impact were formed from sediments which appear to have been only a few tens of metres thick at the time. The implication of this is that an oceanic impact could well

generate tektites simply by sampling thin continentally derived sediments lying on top of the basaltic oceanic crust. In other words, an impact origin of tektites is not precluded by failure to discover a suitable impact structure on land.

In the case of the Australasian strewnfield there are several candidate craters on land — the Zhamanshin and Elgygytyn Craters of the USSR and an unnamed crater-like structure in Cambodia. Nothing much is known of the Cambodian crater and the Elgygytyn Crater can be ruled out on age grounds. The most touted possibility is the Zhamanshin Crater, but the case for it is weak, if not nonexistent. For example, this crater is associated with tektite-like objects known as irghizites, which have chemical compositions similar to, but Nd isotopic compositions quite different from, those of the tektites of the Australasian strewnfield. It is possible in principle that both the irghizites and the Australasian tektites were produced at the same crater but that the irghizites managed to take up a proportion of the impacting meteoritic material, although Shaw and Wasserburg show that it is impossible in practice to obtain the correct ϵ values for the irghizites simply by adding chondritic material to the tektitic. It seems much more likely that the impact body that supposedly gave rise to the Australasian tektites fell in the ocean; but wherever it struck, it generated tektites from continentally derived sediment about 250 million years old.

What is impressive about the story pieced together by Shaw and Wasserburg is its overall coherence and internal consistency. So can we take it that the tektite issue is now settled? Or will the lunar volcanologists fight back, as they have so often done before, with a quite different interpretation of the same data? We shall see. In the meantime, it could well be objected that, coherent or not, the cometary impact hypothesis is no less exotic a mechanism for the production of tektites than is lunar volcanism. But somehow it does seem less so these days. Recent calculations on the frequency with which comets large and small might be expected to reach the Earth's surface, and the remarkably cool way in which the earth science community has been willing to consider seriously the suggestion by Alvarez *et al.* (*Science* 208, 1095; 1980) that cometary impact may have been responsible for the Cretaceous-Tertiary extinctions, suggest that the rigid anti-catastrophism characteristic of geological thinking since the time of Hutton and Lyell is now dead.

Yet at least one puzzle remains, and remains unremarked. If cometary impacts have been responsible for biological extinctions throughout the Phanerozoic, why have they apparently only generated tektites during the past 35 million years? Curious. □

Learning, forgetting and the cell biology of memory

from Miranda Robertson

PERHAPS memory has proved such an intractable problem for so long because, like embryogenesis — the other central metazoan mystery — it is a property of interacting cell systems, depending on cellular signals whose mechanisms seem likely to be the last to yield to molecular analysis. Charles Stevens (Yale University), closing a recent meeting* primarily concerned with the biology of memory (but see box overleaf), argued that memory and development are not merely similar problems but one and the same. From this point of view, the traditional distinction between 'hard-wired' and plastic neuronal systems is reduced to a matter of timing: the final differentiation of some of the neurones in a plastic system is delayed until long after maturity.

Stevens's near-impossible brief was an overall synthesis of a conference that began with the properties of learning and forgetting in damaged and undamaged vertebrates and descended fairly precipitously to the properties of ionic channels in lower forms of life. Paul Rozin (University of Pennsylvania), discussing the adaptive properties of learning at the upper end of this spectrum, gave some arresting examples of plastic systems with a hard-wired basis. The most obvious of these is the imprinting of precocial birds, which follow and later make sexual advances to anything that looks like the first moving object they see (usually their mother); the responses in this case are hard-wired, only the stimulus being determined by experience. More subtle constraints may operate in mammalian learning: for example, a rat taught to associate a light and a specific taste with malaise will learn to avoid the taste, not the light.

One hope of those engaged in research on the neuropsychology of memory is to identify specific brain structures essential for its consolidation. Given that memory can be regarded as a property of any neural circuit whose activity is modifiable by experience, this may seem an optimistic aim. It is therefore perhaps surprising that damage to the medial temporal lobes of the human brain should have proved to be specifically associated with failure of memory for events occurring after the injury, in the absence of sensory or cognitive deficits. In the light of recent analyses, however, the memory deficit begins to look less general than it might at first appear. Brenda Milner (McGill University), for example, summarized recent evidence for lateralization: lesions

of the left medial temporal lobe generally lead to failure of verbal memory, lesions of the right to deficits in memory for spatial position.

A more profound specialization still has been suggested by the research of Neal Cohen in the laboratory of Larry Squire (University of California, San Diego). He has demonstrated that medial temporal lesions affect only what he calls declarative learning — or 'learning that'; damaged patients are still perfectly capable of procedural learning — or 'learning how'. Thus for example, a patient with almost complete failure of verbal memory can be taught to read mirror-reversed writing and retain the skill on testing three months later, though he will not remember having been taught it¹. This is a particularly telling example because reading reversed print is a verbal skill. Squire believes that procedural learning may be different in kind from declarative learning: it takes longer to acquire and is much less easily forgotten, as well as being resistant to brain damage affecting other kinds of memory. Whether this really reflects a difference in kind, or is a function of the number of neural pathways involved, is impossible to say.

Without doubt the most striking and provocative data to be reported at the conference were those of Fernando Nottebohm (Rockefeller University) on song learning in canaries, which is another clear case of partial plasticity. The song of male canaries has an invariant species-specific basis that is elaborated by learning, and the songs of individual birds will change considerably from one year to the next. The ability of the birds to learn new songs (though not their ability to sing) depends on a specialized neural centre known as the hyperstriatum ventrale, pars caudale (HVC). The size of the HVC, and that of a lower song control centre, the nucleus robustus archistrialis (RA), is correlated with the size of the birds' song repertoire — being very small in females, which are songless, and increasing in males during the spring, when testosterone levels are high and the season's song repertoire is growing. In the RA these size differences can be accounted for in part by differences in the size of the dendritic trees of the neurones² and it seems likely that similar changes occur in the HVC.

But recent investigations have suggested the more startling possibility that in the HVC the size fluctuations may be partly due to the continual recruitment and loss of neurones in the nucleus. The evidence for such turnover comes from experiments on female canaries. Under the influence of testosterone, the female HVC doubles in size³. In the light of this dramatic increase,

Nottebohm, with his colleague Steven Goldman, went on to investigate whether testosterone might be inducing cell proliferation and found that new HVC neurones were appearing all the time even in the absence of the hormone. They are accordingly now monitoring the HVcs of both male and female canaries at monthly intervals to see if they can detect changes in the rate of cell division; and Nottebohm suggests that the addition and loss of neurones in the HVC reflects a continuous 'updating of the software' of song production — cell turnover in females reflecting, perhaps, the complementary need to recognize each new season's repertoire of serenades.

The implication of such drastic changes is that learning entails an irreversible change in the HVC neurones so that new learning requires new cells containing uncommitted DNA. If this is the case, there is certainly a radical difference between avian and human song learning; but so little is known of the cellular basis of memory that the possibility cannot be dismissed.

It is generally assumed that learning entails short-term changes in the activity of groups of neurones, leading to longer-term or effectively permanent changes in synaptic resistance. Evidence from the effects of trauma (for example concussion or electroconvulsive shock) suggests that while the short-term changes may have a time course of minutes to hours, longer-term consolidation may continue for up to two years.

Both Eric Kandel (Columbia University) and William Quinn (Princeton University) addressed themselves to the possible molecular basis of these changes in animals with simple nervous systems. Kandel has focused on the modulation of the gill-withdrawal reflex in the sea-snail *Aplysia*. This can be elicited by touching the animal's siphon, and enhanced by simultaneous stimulation of its tail. Kandel has shown that such sensitization, which can be regarded as a simple form of learning, depends on presynaptic modulation by serotonin of the activity of the sensory neurones mediating the reflex. Serotonin binds to receptors on the presynaptic nerve terminal, activating adenylate cyclase and leading to the cyclic AMP-dependent phosphorylation of a potassium channel in the presynaptic membrane. The resultant closure of the potassium channel prolongs the action potential, thus increasing the influx of calcium and the release of transmitter onto the postsynaptic cell^{4,5}. The same mechanism has more recently been shown to underlie a simple form of classical conditioning in which a weak stimulus to the tail precedes the stimulus to the siphon.

Quinn has sought, by the analysis of

*The First McDonnell Conference on Higher Brain Function was held in St Louis on 23–24 September 1982.

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learning mutants, to establish a similar kind of mechanism in *Drosophila*. One mutant, *dunce*, has a defective phosphodiesterase and thus presumably abnormal cyclic AMP metabolism⁶; a second, *rutabaga*, isolated by Patricia Szeiber in his laboratory, has been shown by Margaret Livingstone to be deficient in adenylate cyclase activity. Quinn is now hoping to identify the neurotransmitter whose receptor is (presumably) coupled to the crucial adenylate cyclase, so as to explore further the possible parallel with *Aplysia*. Preliminary evidence implicates either dopamine or serotonin, which is particularly interesting in view of recent evidence that the distribution of dopamine is correlated in rat brain with that of a membrane protein whose phosphorylation can be shown *in vitro* to depend on the dopamine-activated synthesis of cyclic AMP⁷.

Thus catecholamine-activated protein phosphorylation may be part of a general mechanism of memory. But how far can the phosphorylation of ionic channels explain the phenomenological properties of memory? Stevens, in his summary discussion, pointed out that it cannot on its own account for any memory lasting more than two or three weeks, which is the maximum lifetime of a membrane protein. If learning depends on the phosphorylation of membrane proteins, then neurones must be re-programmed to keep phosphorylating them. It was this kind of consideration that led him to suggest that memory can be seen as the last step in neural differentiation: a final change in gene expression that is reserved until after birth and is contingent on experience. Just as during development, a morphogenetic signal changes the state of differentiation of a cell and thus its responses to subsequent signals, so the early changes in neural activity that underlie short-term memory may render the neurone susceptible to later signals that induce a change in gene expression and long-term storage. There is in fact evidence that in some systems, neural morphogenesis is profoundly influenced by neural activity: learning may reflect the same (unknown) mechanism. Indeed changes in dendritic or synaptic morphology may be the basis for long-term memory.

An understanding of memory at this level will not, in Stevens's view, provide a complete understanding of the encoding of memories — any more than understanding the behaviour of transistors would help with the understanding of a computer program. But it could establish, for example, whether the process is reversible or not. It is possible that memories are for ever, and forgetting is just loss of access. Certainly this is to be expected from the developmental parallel, since differentiation is generally extremely stable, if not completely irreversible. There is, however, one notable case in which terminal differentiation entails the editing of the

Seeing in colour

THE showpiece of the First McDonnell Conference on Higher Brain Function was the spectacular re-entry into research on colour vision of David Hubel (Harvard University), who, in a kingfisher shirt and scarlet tie, and with the assistance of Margaret Livingstone manipulating coloured lights, gave a personal demonstration of the importance of context in hue perception. The demonstration is simple (and in principle well known): bathe the outrageous shirt and tie in red light and the outrage vanishes: the shirt becomes black while the tie assumes the same pallid appearance as the speaker's face; similarly in blue light the vividness of the shirt is drained. This illustrates that human colour vision is not a simple function of the wavelength of light reaching the retina — as any reader who has a copy of *Nature* for 3 April 1980 (and has not lost the colour filters supplied with it) can check for himself.

Among the best explanations so far offered for the relative indifference of the visual system to the absolute wavelength of the stimulus is Land's retinex theory (discussed in the same issue of *Nature*¹), according to which the sensation of colour is the outcome of interactions between neurones that respond selectively to lights of different wavelengths. Specifically, the theory predicts the existence of cells whose receptive fields are so organized that they respond maximally when the centre of the field is illuminated by light of a wavelength complementary to that illuminating the surround. A highly effective stimulus would thus be a red dot in a blue surround. Neurones responding to such stimuli are known as double-opponent colour cells and have been detected in very small numbers in the

lateral geniculate nucleus of the cat², which has relatively poor colour vision, and in the visual cortex of the monkey³, whose colour vision is comparable with man's. This anatomical difference between cat and monkey reflects the general rule that sophisticated sensory computations tend to be postponed to increasingly central sites with ascent up the phylogenetic tree.

Hubel and Livingstone have now illuminated a further species difference by locating the cortical double-opponent cells precisely in the patches, or 'blobs', of exceptionally high metabolic activity recently revealed by cytochrome oxidase staining in layers II and III^{4,5}. These patches have two curious features (discussed in *Nature* by Swindale⁶). First, they contain cells that, unlike most visual cortical neurones, are not selective for stimulus orientation⁷; and second, they are absent from the visual cortex of the cat.

The absence of orientation selectivity could be explained if the patches represented areas of convergence of orientation columns⁶ — and thus containing neurones responding to all orientations. But then why none in the cat? A possible answer to this question now suggests itself: the patches may, instead of representing converging orientation columns, be colour columns; and their absence in cats probably reflects the relative crudeness of feline colour vision.

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DNA and irrevocably limits the potential of the cell for further change. This is the case of the antibody-producing lymphocyte, in which recombination of the DNA is part of the basis for antibody diversity. Stevens, echoing Nottebohm's speculations, suggested that some similarly irreversible step might be made by the song cells of the canary HVC — so that the only way to change the repertoire would be by the loss of the old neurones and the addition of new ones.

It is however more likely (as he went on to emphasize) that versatility in memory depends not on the generation of diverse proteins by DNA recombination, but on the versatile deployment of a limited number of related families of receptor and channel proteins whose expression in different combinations confers on nerve

cells the specialized properties of which so far we have such a limited understanding. Now that, with the cloning of the genes encoding the acetylcholine receptor⁸, the first members of these protein families have yielded to recombinant DNA technology, dramatic advances in understanding their relationships and expression may not be far away.

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ARTICLES

Glucocorticoid regulation of protein processing and compartmentalization

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Glucocorticoids appear to regulate the post-translational processing and compartmentalization of certain proteins in cultured rat hepatoma (HTC) cells. We have found that maturation of murine mammary tumour virus (MTV) proteins in infected HTC cells is hormone-dependent. Thus, MTV gene products may represent versatile and sensitive probes for examining cellular regulation of protein maturation.

THE location at which a given protein resides or acts is an integral and important aspect of gene expression. In general, proteins are 'targeted' or transported either during or after synthesis to particular intra- or extracellular compartments; the final gene product may be soluble in the cytoplasm, sequestered within specific intracellular organelles, inserted into particular cellular membranes, or secreted out of the cell. In addition, and perhaps related to this phenomenon of compartmentalization, many proteins are subject to one or more processing or chemical modification reactions including site-specific cleavage, phosphorylation and attachment of carbohydrates or lipids. Thus, the final location and biological activity of such mature proteins could in principle be regulated by modulating the appropriate post-translational events.

What signalling molecules might be involved in regulating gene expression at this level? In animal cells glucocorticoids and other steroid hormones act via specific intracellular receptors to elicit a broad range of responses in various target cells¹⁻³; it has been established in some cases that the hormone-receptor complex directly stimulates transcription of the regulated loci. For example, when DNA encoding MTV is introduced into the genome of HTC cells, dexamethasone, a synthetic glucocorticoid, rapidly and selectively increases the rate of MTV RNA transcription^{4,5}. Dexamethasone also regulates the expression of certain intracellular, cell surface and secreted proteins encoded by the HTC genome⁶⁻¹³; in these cases, the molecular processes under hormonal control have not been fully defined. Glucocorticoids could conceivably affect expression of some genes by stimulating or inhibiting transcription, others at a post-transcriptional event, and still others at multiple stages. To test this notion in detail requires the ability to detect specifically the DNA, RNA and proteins corresponding to a regulated gene and its products; unfortunately, such a combination of molecular hybridization and immunological reagents has not yet been reported for a glucocorticoid-controlled HTC cell gene.

In mammary tumour cells, MTV encodes two major polyproteins, each of which is specifically modified, cleaved and compartmentalized in an array of reactions that appear similar to those that effect maturation of various classes of cellular proteins¹⁴⁻¹⁷; specific antisera and cloned nucleic acid probes are available to monitor the precursor and mature MTV proteins, as well as the viral transcripts and genes. Therefore, MTV gene products in infected cells might be useful as general probes for putative glucocorticoid-regulated cellular functions that act post-translationally. Thus, we have used biochemical and genetic approaches to examine this possibility in an MTV-infected HTC cell derivative.

Effects of dexamethasone on MTV protein expression

Viral proteins were examined in M1.54, a cloned line of infected HTC cells containing 10 stably integrated MTV proviruses. As MTV RNA is produced at a basal rate in this line, and is strongly

induced by dexamethasone^{18,19}, viral protein synthesis and maturation can be examined before and after hormone treatment. In addition, infectious MTV particles are not produced in this heterologous system²⁰, thereby precluding reintroduction of viral proteins into the cells via secondary infection.

In initial experiments, the full complement of MTV proteins produced by these infected rat cells was examined. M1.54 cells were labelled with ³⁵S-methionine for 12 h in the presence or absence of dexamethasone; hormone-treated cultures were exposed to dexamethasone for 24 h before labelling. Cellular and secreted proteins were solubilized in detergent-containing buffer and immunoprecipitated with an anti-MTV antiserum that detects all MTV gene products; to normalize for hormonal induction of viral transcripts, approximately equivalent amounts of immunoprecipitated radioactivity were then analysed on SDS-polyacrylamide gels. In hormone-treated M1.54 cells (Fig. 1a), 13 viral proteins were detected, ranging from molecular weight (M_r) 200,000 to 24,000, and comprising about 2% of the total labelled proteins. As shown below, the 74,000- M_r species actually represents two distinct viral gene products. In contrast, in the control cultures (Fig. 1c), seven of the MTV proteins were barely detectable or absent altogether relative to the remaining species, which were indistinguishable from those produced in dexamethasone-containing medium; the hormone-induced proteins are marked in Fig. 1 with arrows. Control experiments with preimmune sera (Fig. 1b, d) confirmed that all the proteins detected are MTV gene products.

Figure 1 shows that the 12-h labelling period enables even minor viral protein species to be detected; however, the possibility remains that the failure to observe certain of the proteins in the absence of dexamethasone is due to their relative instability, rather than their failure to accumulate. Therefore, cells were pulse-labelled with ³⁵S-methionine for 20 min then incubated for an additional 60- or 120-min 'chase' period in the presence of excess unlabelled methionine. The results (Fig. 2a-f) show that in the absence of dexamethasone, only the 74,000- M_r species is detected in either the pulse-labelled or pulse-chased material; in contrast, the 74,000- M_r protein pulse-labelled in dexamethasone-treated cultures is subsequently chased into the major viral products observed after long-term labelling (compare Figs 1a and 2g-l). While this experiment does not exclude extremely rapid degradation of the proteins in the control cultures, the simplest view is that they are produced only in the presence of hormone. Taken together, these results are consistent with the idea that glucocorticoids in some way regulate post-translational MTV protein maturation in M1.54 cells.

MTV transcripts in M1.54 cells

An alternative explanation for the hormonal effect on MTV protein expression is that dexamethasone-induced MTV transcripts differ qualitatively from those produced in the absence of hormone. Altered patterns of initiation and splicing or polyadenylation have been observed in other systems in response

to changing physiological conditions or differentiation states^{21,22}. By this view, hormonal regulation of transcription would account for qualitative and quantitative changes in MTV protein production.

Thus, MTV RNA was characterized biochemically and functionally. Total RNA from M1.54 cells cultured in the presence or absence of dexamethasone was translated *in vitro* in a rabbit reticulocyte lysate. Immunoprecipitation with anti-MTV antiserum and subsequent electrophoretic analysis revealed no effect of dexamethasone on the three MTV-specific polypeptides synthesized (Fig. 3a–h). In addition, cellular RNA from induced and uninduced cells was fractionated by agarose gel electrophoresis, transferred to nitrocellulose and hybridized with cloned MTV ³²P-DNA; the amount of MTV RNA in each gel sample was normalized. As shown in Fig. 3i, j, dexamethasone has no detectable effect on the sizes or relative stoichiometry of the two MTV RNA species produced in M1.54 cells, whereas no virus-specific sequences are detected in unin-

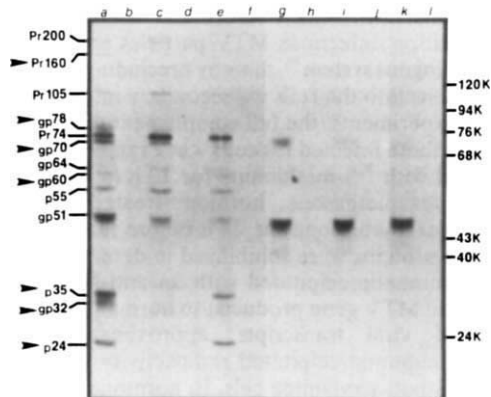


Fig. 1 MTV proteins produced by M1.54, and by a complement-resistant derivative, CR4. M1.54 and CR4 cells (5×10^6) were labelled with $100 \mu\text{Ci}$ ³⁵S-methionine for 12 h in 4 ml methionine-deficient Dulbecco's modified Eagle's medium supplemented with 1% dialysed fetal calf serum; hormone-treated cultures were incubated with $1 \mu\text{M}$ dexamethasone 24 h before as well as during the radiolabelling period. The cells were collected by centrifugation (600g, 3 min), and the supernatant was re-centrifuged (10,000g, 10 min) to remove cell debris. The cell pellets were homogenized in 1 ml of 1% Triton X-100, 0.5% sodium deoxycholate, 5 mM EDTA, 250 mM NaCl, 25 mM Tris-HCl, pH 7.5 (solubilization buffer) and the solubilized proteins collected as the supernatant after centrifugation at 20,000g for 20 min at 4°C. The medium was adjusted to 1% Triton X-100, 0.5% sodium deoxycholate, 5 mM EDTA just before immunoprecipitation. Viral proteins were immunoprecipitated by a modification of the staphylococcal protein A-antibody adsorption procedure⁴¹; 10-fold more extract from uninduced cells was immunoadsorbed, thereby approximately equalizing the amounts of MTV proteins analysed per sample. The detergent-solubilized fractions (300–500 μl) were preadsorbed with $10 \mu\text{l}$ of 10% fixed *Staphylococcus aureus* (Staph A) for 15 min at room temperature. After removal of Staph A by centrifugation, $1 \mu\text{l}$ of either anti-MTV rabbit serum (lanes a, c, e, g, i, k) or preimmune serum (lanes b, d, f, h, j, l) was added; antisera were given by R. D. Cardiff and co-workers⁴². The mixtures were incubated at room temperature for 15 min before addition of $10 \mu\text{l}$ of 10% fixed Staph A that had been preadsorbed with unlabelled cell extracts. After incubation for 5 min, the Staph A was centrifuged through 1 M sucrose in solubilization buffer, washed once in solubilization buffer and then once in 5 mM EDTA, 10 mM Tris-HCl, pH 7.5 (buffer B). Nonspecific binding of cellular radiolabelled proteins was significantly reduced by a second immunoprecipitation of the viral antigens. The immunoadsorbed antigens were eluted from the Staph A-associated IgG by incubating the Staph A pellets with $30 \mu\text{l}$ of 2% SDS at 37°C for 20 min. After removing the Staph A by centrifugation, the eluted viral antigens were added to 400 μl of solubilization buffer containing 0.8 μl of antiserum and incubated at room temperature for 15 min. Fixed Staph A ($10 \mu\text{l}$ of a 10% suspension) was added, incubated for 5 min and collected by centrifugation. The pellets were washed once in solubilization buffer and once in buffer B. Finally, the immunoadsorbed MTV proteins were eluted from the Staph A in SDS sample buffer, fractionated by SDS-polyacrylamide gel electrophoresis⁴³, and visualized by fluorography⁴⁴. The figure shows cellular MTV proteins from hormone-treated M1.54 (a, b), uninduced M1.54 (c, d) and hormone-treated CR4 (e, f). Arrows denote the viral gene products produced in a hormone-dependent manner in M1.54. Also shown are secreted MTV proteins from hormone-treated M1.54 (g, h), uninduced M1.54 (i, j) and hormone-treated CR4 (k, l). The molecular weight markers are: β -galactosidase (M_r 120,000; 120K), phosphorylase a (94K), conalbumin (76K), bovine serum albumin (BSA; 68K), ovalbumin (43K), aldolase (40K) and chymotrypsinogen (24K).

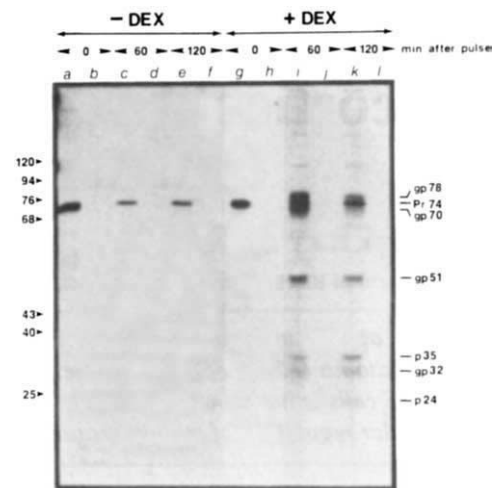


Fig. 2 ³⁵S-methionine MTV proteins produced by M1.54 during pulse and subsequent chase. M1.54 cells (10^7) were labelled at 37°C for 20 min in medium containing $100 \mu\text{Ci ml}^{-1}$ ³⁵S-methionine; cultures were either collected immediately or incubated for an additional 60 or 120 min in chase medium containing 2 mM unlabelled methionine. Hormone treatment, preparation of extracts, immunoprecipitation, gel electrophoresis and molecular weight standards were as described in Fig. 1 legend. Immunoprecipitation was with anti-MTV rabbit serum (lanes a, c, e, g, i, k) or preimmune serum (lanes b, d, f, h, j, l). a–f, Cellular MTV protein from uninduced M1.54 cells that were: a, b, pulse-labelled for 20 min; c, d, pulse-labelled and chased for 60 min; and e, f, pulse-labelled and chased for 120 min. g–l, MTV proteins from dexamethasone-treated M1.54 cultures that were: g, h, pulse-labelled for 20 min; i, j, pulse-labelled and chased for 60 min; and k, l, pulse-labelled and chased for 120 min.

ected HTC cells (Fig. 3k, l). Further experiments have established that the hormone does not affect the site of initiation of viral RNA transcription in infected cells, nor does it alter either the sites or efficiencies of MTV RNA splicing and polyadenylation²³. Taken together, these results show that the MTV RNA molecules produced in the presence and absence of dexamethasone in M1.54 cells are qualitatively and functionally identical, and that any effect of glucocorticoids on viral protein maturation probably occurs co- or post-translationally. Independent support for this view is provided by experiments demonstrating that an MTV precursor protein pulse-labelled with ³⁵S-methionine in untreated M1.54 cells is subsequently matured when dexamethasone is added to the medium during the chase period (G.L.F., unpublished result).

Regulation of MTV phosphoprotein maturation in M1.54 cells

Two MTV transcripts similar to those described here are detected in virion-producing mouse mammary tumour cell lines; one specifies core proteins encoded by the *gag* gene, while the other specifies envelope proteins encoded by the *env* gene²⁴. Each precursor acquires characteristic modifications; the *gag* polypeptides are phosphoproteins whereas the *env* polypeptides are glycoproteins. In both cases, the polypeptides are subsequently processed into several mature species. Thus, monospecific antibodies directed against either the MTV envelope glycoproteins or the core phosphoproteins were used to identify further the MTV proteins produced in M1.54 cells. This showed that the mature *gag*-encoded species are p55, p35 and p24, and that the mature *env*-encoded species are gp78, gp70, gp64, gp60, gp51 and gp32 (see Fig. 1 and below). In addition, the major precursors encoded by both the *gag* and *env* genes are M_r 74,000, and are designated as Pr74 in Fig. 1.

Immunoprecipitation with the monospecific antibodies also revealed that all the viral phosphoproteins in M1.54 are *gag*-related (data not shown); therefore, ³²P-phosphate was used to label selectively the *gag* proteins. Figure 4 shows that

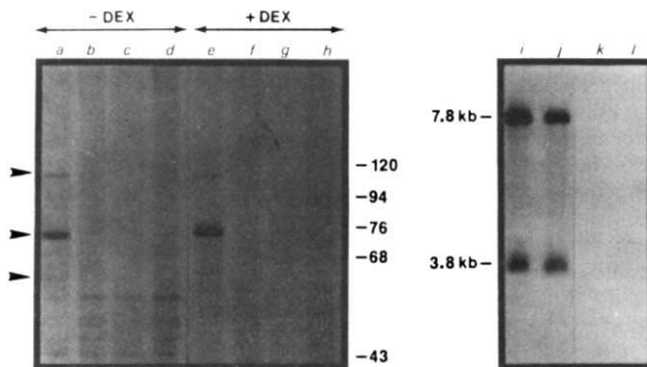


Fig. 3 Functional and biochemical analysis of MTV RNA. Total RNA, isolated from control or dexamethasone-treated ($1 \mu\text{M}$, 24 h) cultures of M1.54 and HTC by a combination of the guanidinium thiocyanate cell lysis⁴⁵ and CsCl centrifugation⁴⁶ methods, was subjected to *in vitro* translation (a–h) and size fractionation (i–l). For cell-free protein synthesis, typical 50- μl reaction mixtures contained 40 μCi ^{35}S -methionine ($\sim 1,000 \text{ Ci mmol}^{-1}$), 35 μl of nuclease-treated rabbit reticulocyte lysate^{47,48} and 10 μg of total RNA. After 90 min at 37°C , the incubation mixtures were added to 400 μl solubilization buffer, immunoprecipitated and fractionated in SDS gels together with molecular weight standards as in Fig. 1. Immunoprecipitations were carried out with anti-MTV serum (a, c, e, g) or preimmune serum (b, d, f, h); RNA was from uninduced M1.54 (a, b) and HTC (c, d) cells, and from hormone-induced M1.54 (e, f) and HTC (g, h) cells. Immunoprecipitations were done using 10-fold more translation products from uninduced cell RNA in order to approximately equalize levels of immunoprecipitated MTV polypeptides. Arrows denote MTV-specific *in vitro* translation products. RNA was size-fractionated by electrophoresis in formaldehyde-agarose gels⁴⁹; 10-fold more total RNA from uninduced cells (5 μg) was analysed relative to RNA from hormone-induced cells (0.5 μg). RNA was heated to 68°C for 5 min in 50% formamide, 2.2 M formaldehyde in running buffer (1 mM EDTA, 5 mM sodium acetate, 10 mM sodium phosphate, pH 7.0), then electrophoresed in 0.8% agarose gels containing 2.2 M formaldehyde in running buffer. The fractionated RNA was transferred to nitrocellulose filters in $20\times\text{SSC}$ ($1\times\text{SSC}$ is 150 mM NaCl, 15 mM sodium citrate), and the filters were baked at 80°C for 2 h *in vacuo* and incubated at 42°C for 16 h in annealing mix [$3\times\text{SSC}$, 50% formamide, 50 mg ml^{-1} sheared denatured salmon sperm DNA, $5\times\text{Denhardt's}$ solution ($1\times\text{Denhardt's}$ is 0.02% (w/v) each of BSA, polyvinyl pyrrolidone and Ficoll)]. The filters were then hybridized (18 h at 42°C) with cloned MTV ^{32}P -DNA ($1\text{--}3\times 10^8 \text{ d.p.m. } \mu\text{g}^{-1}$) in annealing mix containing 10% dextran sulphate, washed three times for 20 min in $0.1\times\text{SSC}$, 0.1% SDS at 50°C and subjected to autoradiography at -70°C . i, RNA from hormone-treated M1.54; j, RNA from uninduced M1.54; k, RNA from hormone-treated HTC; and l, RNA from uninduced HTC cells.

dexamethasone significantly alters viral phosphoprotein maturation in M1.54. The predominant phosphorylated species produced in the absence of dexamethasone is Pr74^{agg}; hormonal treatment results in the production of low levels of a second phosphorylated gag polyprotein, p76, and two presumptive maturation products, p35 and p24. The constitutively produced gag product, p55 (Fig. 1a, c), is not detectably phosphorylated. One interpretation of these data is that dexamethasone potentiates the proteolytic cleavage of one or both phosphorylated gag polyproteins into two smaller phosphorylated species; alternatively, the hormone might stimulate Pr74 phosphorylation to the p76 species, thereby permitting subsequent cleavage.

Two phosphorylated MTV gag polyproteins have also been detected in a line of mouse thymic lymphoma cells. In this case, it was found that phosphatase digestion of the larger species yielded the smaller product, implying that the larger species may reflect additional phosphorylation of the smaller²⁵. We have not yet determined whether the dexamethasone-induced appearance of p76 in M1.54 cells involves a second phosphorylation of Pr74, or whether p76 is required for the apparent specific proteolytic processing of the polyprotein.

Regulation of MTV glycoprotein maturation in M1.54 cells

As covalent attachment of mannose-containing glycosylamine-linked oligosaccharide side chains is one of the initial steps in cellular glycoprotein synthesis, [$2\text{--}^3\text{H}$]mannose provides a sensitive tracer for monitoring MTV glycoprotein maturation²⁶. The time course of the process was examined by exposing control and hormone-induced M1.54 cultures to [$2\text{--}^3\text{H}$]mannose for

20 min, followed by additional incubation in the presence of excess unlabelled mannose. At various times during this pulse-chase, the viral proteins were immunoprecipitated with anti-MTV antiserum, fractionated by electrophoresis and visualized by fluorography.

Figure 5a, c shows that M1.54 cells initially synthesize a 74,000-M_r precursor glycoprotein, Pr74^{env}, both in the presence and absence of dexamethasone. After a 150-min chase period, the hormone-treated cells (Fig. 5b) appear to have processed Pr74^{env} into each of the known glycoprotein products (gp78, gp70, gp64, gp60, gp51 and gp32), while a stable form of Pr74^{env} persists. In contrast, the chased control cultures (Fig. 5d) produce very low levels of gp78 relative to Pr74^{env}, and yield no detectable gp70, gp60 or gp32; significantly, the uninduced cells do produce gp51 as an apparent Pr74^{env} processing product. Thus, Pr74^{env} seems to be processed in at least two distinct ways: a constitutive reaction giving a single product, and a hormone-inducible pathway resulting in a more complex mixture of species.

Localization of MTV glycoproteins was examined in separate experiments. Plasma membrane proteins were labelled by lactoperoxidase-mediated iodination of intact hormone-treated M1.54 cells; immunoprecipitation revealed that gp78, gp70, gp51 and gp32 are located at the cell surface²⁷. When ^{35}S -methionine-labelled extracellular proteins were reacted with this same antiserum, we observed that hormone-treated cells secreted gp51 and gp70 (Fig. 1g, h); glycosylation of the secreted viral species was confirmed by immunoprecipitation of [$2\text{--}^3\text{H}$]mannose-labelled extracellular proteins (data not shown). In fully induced M1.54 cells, the secreted gp51 and gp70 represent $\sim 90\%$ of the MTV glycoproteins that accumulate over a 24-h period. Figure 1 l, j shows that uninduced cells secrete much lower levels of gp70 than gp51. Thus, gp51 maturation seems hormone-independent while the production and secretion of gp70 is glucocorticoid-regulated.

Isolation of mutants defective in MTV protein maturation

Our results are consistent with the view that dexamethasone affects a spectrum of protein maturation reactions that alter

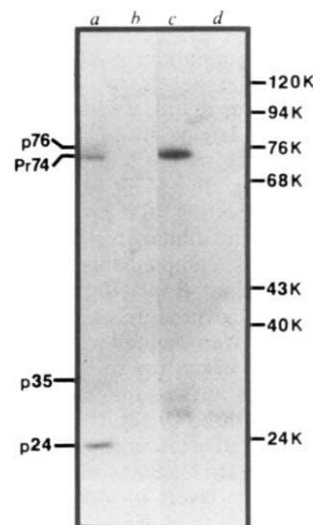


Fig. 4 Glucocorticoid-dependent maturation of MTV phosphoproteins. Dexamethasone-treated or control cultures of M1.54 were labelled with 1 mCi ^{32}P -phosphate for 12 h in phosphate-deficient medium; dexamethasone treatment was as described in Fig. 1 legend. The cell-associated MTV proteins were detergent-solubilized, immunoprecipitated and analysed as described in Fig. 1 legend; approximately 10-fold more extract from uninduced cells was used in the immunoprecipitations. a, Hormone-treated cells and anti-MTV serum; b, hormone-treated cells and preimmune serum; c, uninduced cells and anti-MTV serum; d, uninduced cells and preimmune serum. Molecular weight standards are as in Fig. 1.

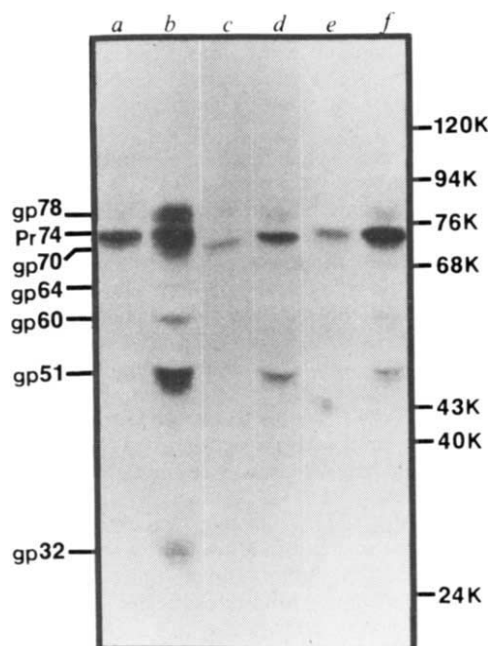


Fig. 5 MTV glycoprotein expression in M1.54 and CR4 cells. Cells were pulse-labelled at 37 °C for 20 min with 200 μ Ci [2- 3 H]mannose and then incubated in the presence of 25 mM unlabelled mannose for further 150 min; hormone treatment was as described in Fig. 1 legend. Cell pellets were detergent-solubilized, immunoprecipitated and the viral glycoproteins analysed as described in Fig. 1 legend. Approximately 10-fold more material from uninduced cells was used for the immunoprecipitations. All lanes show immunoprecipitations with anti-MTV serum; parallel immunoprecipitations with preimmune serum demonstrated that all the glycoproteins detected are MTV-specific (data not shown). *a*, Pulse-labelled hormone-induced M1.54; *b*, 150 min chase of *a*; *c*, pulse-labelled uninduced M1.54; *d*, 150 min chase of *c*; *e*, pulse-labelled hormone-induced CR4; *f*, 150 min chase of *e*. Molecular weight standards are as in Fig. 1.

the efficiency of phosphorylation, glycosylation, cleavage and compartmentalization of a specific class of proteins. To examine these processes in greater detail, we have isolated mutant cell lines carrying genetic defects in MTV protein maturation. M1.54 was chosen as the parent line in our initial selections; this line carries 10 integrated MTV proviruses, thus it seems likely that mutations affecting viral protein maturation in this line will reflect lesions in cellular rather than viral sequences.

For these experiments, complement-mediated cytotoxicity was used to select mutants not displaying hormone-induced cell surface-associated MTV glycoproteins. M1.54 cells exposed to dexamethasone for 24 h were treated with anti-MTV antiserum and rabbit complement according to the method of Kabat *et al.*²⁸; cells carrying viral surface antigens are rapidly lysed by the complement. After three cycles of selection and regrowth of surviving cells, the putative mutants were cloned and isolated.

One such complement-resistant mutant, CR4, expresses approximately wild-type levels of hormone-inducible MTV RNA²⁷, but is defective in the hormone-induced processing of Pr74^{env}. In the presence of dexamethasone, MTV glycoprotein processing in CR4 seems similar to the uninduced parent M1.54 cells; thus, CR4 cells essentially lack intracellular gp78, gp70 and gp32 (Fig. 1*e, f*; Fig. 5*e, f*), as well as secreted gp70 (Fig. 1*k, l*), whereas gp51 is produced competently and secreted. Interestingly, MTV phosphoprotein maturation seems to be unimpaired in CR4 cells (Fig. 1*e, f*). This genetic uncoupling of the glucocorticoid-dependent processing of phosphoproteins and glycoproteins is consistent with the view that at least two distinct protein maturation events may be hormonally regulated in HTC cells.

Conclusions and implications

Dexamethasone has striking effects on the expression of intracellular and extracellular MTV proteins in M1.54; Fig. 6 summarizes our view of the overall pattern of maturation. The dexamethasone-mediated stimulation of MTV RNA production suggests a trivial explanation for our observations: the precursor polypeptide levels may simply be increased sufficiently to allow their recognition by pre-existing maturation enzymes. However, a complement-resistant mutant of M1.54 to be described elsewhere²⁷ argues against this possibility, as MTV RNA and precursor protein levels are not increased by dexamethasone in that line, yet the cell surface glycoproteins are nevertheless matured.

Assuming that the processing and compartmentalization of the viral proteins is mediated by cellular components, these results suggest that glucocorticoid hormones may regulate HTC cell gene expression post-translationally. Conceivably, this mode of control by glucocorticoids could be of general significance for steroid hormonal regulation of developmental and physiological processes. It has been clearly established in a few cases that steroids affect the rate of specific mRNA transcription or turnover^{4,5,29-31}. The present work now implies that these same signalling molecules can dramatically affect the expression of a gene long after it has been transcribed. In this regard, it is interesting to note that dexamethasone regulates the expression of MTV gene products in M1.54 cells both at the transcriptional and post-transcriptional levels.

Our genetic and biochemical evidence suggests that at least two post-translational maturation pathways are glucocorticoid-regulated, one controlling glycoprotein processing and compartmentalization, and another involved in phosphoprotein maturation. Recent results indicate that MTV glycoprotein maturation seems similar to that described for certain cellular proteins and for the G-protein of vesicular stomatitis virus³². Thus, Pr74^{env} contains incomplete oligosaccharide side chains, as they are cleaved by endo- β -*N*-acetylglucosaminidase H (endo H)³³, while its secreted or plasma membrane-associated maturation products (gp78, gp70, gp51 and gp32) contain complete oligosaccharide side chains, as judged by their resistance to endo H digestion³³. In addition, the cell surface forms of

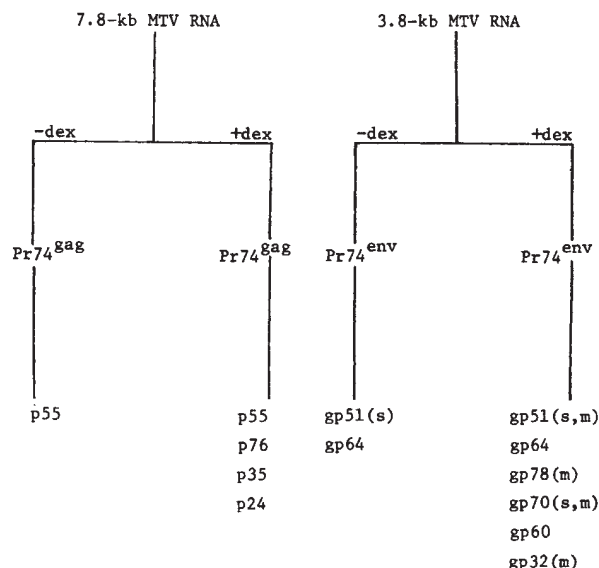


Fig. 6 Proposed pathways of MTV protein maturation in M1.54 cells. The *gag*-encoded precursor polypeptide is translated from 7.8-kilobase (kb) MTV RNA^{24,50} and is differentially processed in the presence (+dex) and absence (-dex) of dexamethasone. Similarly, the *env*-encoded precursor glycopolypeptide is translated from 3.8-kb MTV RNA^{24,46} and then processed in a hormone-dependent manner. s, Secreted glycoproteins; m, plasma membrane-associated species. Several larger precursors (Pr200, Pr160, Pr105) are also detected (see Fig. 1); these minor species are not shown in this diagram.

gp78, gp70 and gp32 are modified by the attachment of palmitic acid (G.L.F., unpublished result).

Clearly, there are many potential targets for hormone regulation of protein maturation. For example, glycosyltransferases, glycosidases, proteases or protein kinases that recognize the viral proteins as substrates could be hormone-induced at transcriptional or post-transcriptional levels. In fact, steroid hormones have been reported to alter the apparent activities or concentrations of such enzymes, but specific substrates and direct molecular and genetic approaches were not available in these cases³⁴⁻³⁸. Regulation of protein processing activities could affect not only the 'targeting' of proteins to particular locations, but might also change the stability of the polypeptide precursors. Another possibility is that dexamethasone alters the expression of 'compartmentalization factors' analogous to those involved in intracellular membrane localization of β -glucuronidase in kidney epithelia³⁹, or to β_2 -microglobulin-mediated membrane insertion of histocompatibility locus antigens⁴⁰.

In any case, it is apparent that glucocorticoids are capable of regulating post-translational aspects of gene expression. These hormonal effects were detected in the present studies by

exploiting MTV gene products as specific probes within HTC cells. As MTV appears to infect cultured cells from a broad range of species and tissue types, it may be possible to use these genes to test the generality of these protein maturation regulatory phenomena. Recently, we have detected the dexamethasone-dependent production of an MTV phosphoprotein in GR cells, a virion-producing mouse mammary carcinoma cell line (G.L.F., unpublished result). Given these sensitive molecular probes, it may be possible to elucidate the mechanisms by which glucocorticoids affect protein maturation.

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Recombination between dispersed serine tRNA genes in *Schizosaccharomyces pombe*

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During meiosis in fission yeast, transfer of genetic information occurs between tRNA genes of related sequence even when they are located on different chromosomes. This process of intergenic conversion has been analysed with the help of nonsense suppressors derived from three unlinked serine tRNA genes. In their wild-type form two of the genes code for tRNA^{Ser}_{UCA}, the third for tRNA^{Ser}_{UCG}. We demonstrate the transfer of anticodon and adjacent intron sequences between two tRNA genes coding for different serine isoacceptors.

THE first gene families studied in detail were the tandemly arranged ribosomal RNA genes. To explain the striking sequence conservation between individual copies in a given species, several rectification models have been proposed¹. One of these mechanisms, unequal crossing-over, has been documented experimentally for the rRNA genes in *Saccharomyces cerevisiae*^{2,3} and *Salmonella typhimurium*⁴. Meanwhile, it has been found that rectification mechanisms must also be respon-

sible for the concerted evolution⁵ observed in other gene families which are not necessarily arranged in tandem, such as the repeated globin genes⁶ and immunoglobulin genes⁷. Further examples include separated blocks of tandemly repeated genes that are located on non-homologous chromosomes, that is, rRNA genes^{8,9} and repetitive sequences of unknown function¹⁰. Finally, concerted evolution has also been observed among the completely dispersed 5S rRNA genes in *Neurospora*

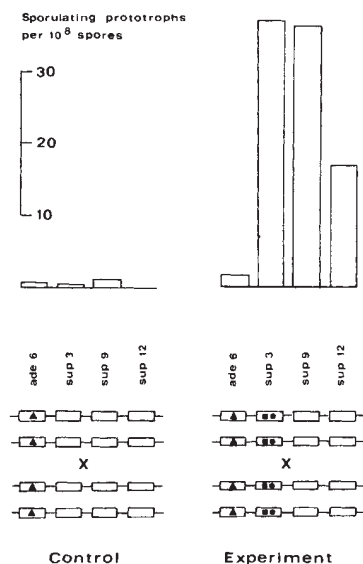


Fig. 1 Frequencies of prototrophs in experimental selfings and controls. The contrasting results obtained from the control cross no. 13 and the experimental cross no. 15 are shown (see Table 1). The gene symbols apply to both the upper and lower parts of the figure. The lower part depicts the crosses between diploid parents. This schematic representation does not reflect the known linkage relationships between the genes concerned. In both cases all *ade6* loci were occupied by a suppressible nonsense allele (*ade6-704*, Δ). The only difference between the two crosses concerns the *sup3* genes: in the control all were wild type; the experimental cross was homozygous for the *sup3-UGA, r36* allele, combining suppressor active anticodon information ($\blacksquare$) with a secondary inactivating lesion (*r36*, $\bullet$). All other suppressor loci were occupied by wild-type alleles in both crosses. The upper part gives the frequency of sporulating prototrophic revertants owing their prototrophy to a reversion of the *ade6* mutant gene or to an active suppressor as indicated. Overall frequencies would be 50% higher because only sporulating revertants heterozygous for mating type have been analysed. We assume that the pattern of events is the same for the sporulating (2/3) and the non-sporulating (1/3) population, but under any assumption the marked increase of *sup3-UGA, sup9-UGA* and *sup12-UGA* events in the experimental situation, compared with the control, would persist.

*crassa*¹¹. Molecular mechanisms proposed to contribute to the concerted evolution not only of tandemly arranged but also of dispersed repeated genes include gene transposition¹¹ and intergenic conversion^{6,7,9,12,13}.

Intergenic conversion implies an information transfer between nonallelic genes of related nucleotide sequence by a mechanism analogous to that proposed for recombination of allelic genes¹⁴. Within a family of directly repeated genes, intergenic conversion without associated crossing-over does not lead to copy-number variation. Therefore, gene duplications and deletions which are the consequence of models based solely on unequal crossing-over do not necessarily occur during concerted evolution. In the case of dispersed repeated genes, intergenic conversion may well be the main mechanism permitting co-evolution. That intergenic conversion really occurs in mitosis and meiosis has been demonstrated in *S. cerevisiae* for pairs of mutationally inactivated protein structural genes¹⁵⁻¹⁷. In these studies a second copy of a given gene was integrated at various sites in the genome by transformation and in those cases in which the two genes carried different mutations, fully active genes were obtained by recombination. The two cytochrome *c* genes in *S. cerevisiae* are examples of naturally occurring genes with related sequences mapping on different chromosomes. In this case, DNA sequencing of revertants of several mutations revealed the import of blocks of wild-type sequence from the corresponding non-identical gene¹⁸.

Transfer RNA genes are widely dispersed in the genomes of most organisms. Nevertheless, multiple genes coding for a specific isoacceptor very often maintain one common nucleotide sequence in the segments that code for the mature tRNA. We previously reported genetic evidence that intergenic conversion occurs between dispersed serine tRNA genes in *Schizosac-*

*charomyces pombe*¹⁹. Here we present further genetic and physical data indicating that information transfer occurs spontaneously during meiosis between three unlinked serine tRNA genes that code in their wild-type form for two different serine isoacceptor tRNAs. We show that the different anticodons and also adjacent diverging sequences of the introns may be exchanged between these tRNA genes.

The genes *sup3* on chromosome I and *sup9* on chromosome III are the only ones that in their wild-type form encode the UCA reading isoacceptor with the anticodon U*GA (where U* is a modified uridine)²⁰. Both have been sequenced recently (ref. 21 and D. Söll, personal communication) and are also available as opal (UGA) suppressor alleles coding for tRNAs with the anticodon U*CA²². The single gene *sup12* on chromosome I codes in its wild-type form for the UCG reading serine tRNA with the anticodon CGA and has been sequenced earlier²³. All three genes are organized in a unique way (Fig. 4): the serine tRNA coding sequences are identical with the exception of one differing base pair corresponding to the tip of the tRNA extra loop and of the obvious differences between the anticodon sequences. The genes *sup3* and *sup9* (tRNA^{Ser}_{UCA}) carry identical introns of 15 base pairs (bp) which differ at six positions from the 16-bp intron of *sup12* (tRNA^{Ser}_{UCG}). On the 3' side of each of these serine tRNA genes follows a completely conserved DNA sequence comprising a spacer of 7 bp and a gene coding for initiator tRNA^{Met}. Thus, the region of sequence homology between the three DNA segments is ~200 nucleotides long including a few partially conserved base pairs in the flanking regions.

Informational transfer between non-linked serine tRNA genes is documented genetically by comparing two types of crosses. In type I, the control, the two parents are identical, except for mating-type, and carry a nonsense mutation in a

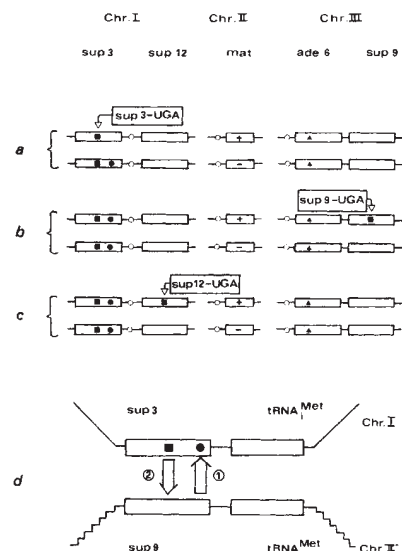


Fig. 2 Import and export of genetic information by intergenic conversion.

The genotypes of the predominant classes of prototrophs obtained from cross no. 15 are depicted. *sup3* and *sup12* are on chromosome I, yet genetically not closely linked with each other. *mat* refers to the mating-type locus on chromosome II. *ade6* and *sup9* are located on chromosome III²⁶. Empty rectangles represent wild-type genes, symbols as in Fig. 1. *a-c*, Prototrophic diploids owing their adenine independence to one copy of the dominant and efficient suppressors *sup3-UGA*, *sup9-UGA* and *sup12-UGA*, respectively. *d*, A schematic representation of the mechanism thought to generate the vast majority of these prototrophic revertants. For example, the wild-type gene *sup9*⁺ may interact during meiosis with the related partner *sup3-UGA, r36*. A single-site conversion replacing the inactivating *r36* site, but not the anticodon site, of the doubly marked *sup3* gene by a corresponding wild-type segment from *sup9*⁺ leads to an active *sup3-UGA* allele (1). On the other hand, transfer of the suppressor active anticodon sequence, divorced from *r36*, to the wild-type gene *sup9*⁺ will generate a *sup9-UGA* suppressor (2). As in conversion involving allelic genes the donating partner is found to emerge unchanged from the interaction. Entirely analogous events are postulated to occur also between *sup3-UGA, r36* and *sup12*⁺.

biosynthetic gene. They are thus auxotrophic. In type II, the experimental situation, the parents carry in addition an identical inactive suppressor allele at corresponding loci. These alleles have suppressor-active anticodon information (obtained by mutation in a first step) but are prevented from suppressing the background nonsense mutation due to a secondary intragenic lesion (obtained in a second mutational step). They are thus also auxotrophic. In both cases prototrophic revertants can be obtained by plating progeny spores on media lacking the growth factor whose biosynthesis is prevented by the nonsense mutation. Type I gives few revertants which result from reversion of the nonsense mutation and from forward mutations at suppressor loci in comparable frequencies. This represents the mutational background. Type II shows a dramatic increase in reversion frequency. The surplus events occur both in the suppressor gene that was originally doubly marked in the parents of the cross and in the two remaining serine tRNA genes which were originally present in wild-type form. Mutation cannot explain this result. Also, recombination between the doubly-marked suppressor genes of the parents does not lead to an active suppressor because these genes are identical. Instead, we propose that non-allelic members of this gene family may interact and exchange information in a process analogous to gene conversion between allelic genes. Non-reciprocal information transfer may occur in either direction when the doubly-marked gene interacts with a wild-type member of the family: replacement of the secondary lesion by wild-type information leads to an active suppressor in the former, transfer of the suppressor-active anticodon information into the wild-type

gene leads to an active suppressor in the latter. Physical analysis has corroborated this interpretation, as restriction mapping and DNA sequencing show that genes obtained by this process of intergenic conversion may differ from the original copy at several non-adjacent sites, reflecting the transfer of DNA segments.

Two known suppressor tRNA genes import information from non-allelic genes

To detect rare intergenic convertants an experimental system is needed that allows discrimination against events caused by recombination between allelic genes. This we have achieved by carrying out homozygous crosses, that is, selfings of strains carrying inactivated suppressors.

A first series of experiments involved the selfing of auxotrophic haploid strains and the analysis of the rare prototrophic revertants obtained among the progeny spores. The results are summarized in the first part of Table 1 (expts 1–12). Strains carrying either the UGA nonsense mutation *ade6-704* or *ade1-40* in combination with wild-type genes at all suppressor loci served as controls (expts 1 and 8). The experimental strains carried, on the background of one or the other of these nonsense mutations, inactive suppressor alleles combining suppressor active anticodon information with a secondary inactivating lesion²⁴ (*sup3-UGA, rX*: expts 2–7; *sup9-UGA, rX*: expts 9–12). It is obvious that the reversion frequency is much higher in the experimental selfings than in the controls. Furthermore, the prototrophy of the revertants obtained from the experi-

Table 1 Frequency and nature of prototrophic revertants from selfings

Expt. no.	Ploidy of strains	Suppressor allele involved	No. of independent expts	Prototrophic revertant colonies observed	Total spores analysed ($\times 10^8$)	Reversion frequency ($\times 10^{-8}$)	Nature of prototrophic revertants						Total revertants analysed
							<i>ade</i> ⁺	<i>sup3-UGA</i>	<i>sup9-UGA</i>	<i>sup12-UGA</i>	<i>sup8-UGA</i>	<i>sup10-UGA</i>	
1	n	<i>sup3</i> ⁺ (UCA)	3	14	9.0	2		10	3		1		14
2	n	<i>sup3-UGA, r8</i>	3	319	10.1	32		76				3	79
3	n	<i>sup3-UGA, r10</i>	3	2474	12.0	206		73				1	74
4	n	<i>sup3-UGA, r15</i>	3	158	11.7	14		63				15	78
5	n	<i>sup3-UGA, r30</i>	3	955	9.9	96		58			1	4	63
6	n	<i>sup3-UGA, r36</i>	3	914	10.2	90		80					80
7	n	<i>sup3-UGA, r40</i>	3	819	15.6	53		75				4	79
8	n	<i>sup9</i> ⁺ (UCA)	3	17	5.3	3	3	5	9				17
9	n	<i>sup9-UGA, r49</i>	3	1950	16.9	115			58		1	5	64
10	n	<i>sup9-UGA, r68</i>	3	987	15.3	65			68			2	70
11	n	<i>sup9-UGA, r101</i>	3	602	13.5	45			73		1	4	78
12	n	<i>sup9-UGA, r104</i>	3	713	6.5	110			64		1	2	67
13	2n	<i>sup3</i> ⁺ (UCA)	6	10	2.7	4	2	1	3			1	7
14	2n	<i>sup3-UGA, r8</i>	4	38	1.5	25		10	4	2		1	17
15	2n	<i>sup3-UGA, r36</i>	4	198	1.3	152	2	48	47	22			119

Strains 1–7 are of general constitution $h^{90} ade6-704 sup3$ and strains 8–12 of constitution $h^{90} ade1-40 sup9$ with the particular suppressor alleles indicated. They are haploid and homothallic, that is, self-sporulating. In sporulating conditions they produce diploid zygotes leading on meiosis to asci containing four haploid and homothallic spores. Thus, this procedure is equivalent to crossing two heterothallic strains differing only at the mating-type locus (h^+ and h^-). *ade6-704* and *ade1-40* are both suppressible UGA nonsense mutations which lead, when not suppressed, to an adenine requirement and, in the case of *ade6-704*, to the formation of a characteristic red pigment in colonies grown on media with low adenine concentrations such as yeast extract agar. Crosses 13–15 involve two diploid parents each and are of the general type $(h^+/h^+ ade6-704/ade6-704 sup3/sup3) \times (h^-/h^- ade6-704/ade6-704 sup3/sup3)$ with particular *sup3* alleles indicated. The two parents of any of these three crosses are different only with respect to the mating-type locus. Such matings result in tetraploid zygotes which after meiosis produce asci with four diploid spores. Two-thirds of these spores are of heterozygous mating-type constitution (h^+/h^-) and give rise to diploid clones, which in turn will sporulate in appropriate conditions producing haploid progeny. They are directly amenable to genetic testing, in contrast to the last third which is stable and of constitution h^+/h^+ and h^-/h^- , respectively. The suppressor alleles involved in these selfings are either wild-type or double-mutant alleles combining suppressor active anticodon information with a secondary inactivating lesion elsewhere in the suppressor gene²⁴. Selfings 1, 8 and 13 represent controls and are alike in the sense that only wild-type alleles are involved at all suppressor loci concerned. Selfings were done on malt extract agar slants supplemented with adenine (8 days, 25 °C). The sporulated cultures were suspended in water, treated overnight with snail digestive juice to inactivate remaining vegetative cells, washed and resuspended in 5 ml water. 4 ml of each suspension were distributed on 20 minimal agar plates in order to select prototrophic revertants (selfings 1–12: 7 days, 30 °C; selfings 13–15: 9 days, 25 °C). Aliquots of appropriate dilutions were spread on yeast extract agar for total spore counts. The number of independent repeats of a particular selfing is indicated. Pooled data are given as no cases of extreme fluctuation were observed. A sample of the revertants was analysed to determine the factor responsible for prototrophy. In the case of selfings 2–7 and 9–12 some 70 randomly chosen revertants were analysed per selfing. These were taken in approximately equal numbers from the three repeats. The sample in selfings 13–15 consisted of all sporulating revertants obtained. The nature of these revertants was assessed in crosses with a set of five haploid tester strains: a wild-type strain and four mutant strains each of which carried one of four efficient suppressors on the background of *ade6-704*. The haploid revertants from selfings 1–12 could be directly crossed to the testers. From the sporulating diploid revertants of selfings 13–15, 8 tetrads were analysed per revertant strain. Class I isolates produced two prototrophic and two adenine-dependent segregants per tetrad. One prototrophic haploid segregant was used for testing. Class II isolates were observed only in selfings 14 and 15 and gave two lethal and two auxotrophic segregants per tetrad. They could be subdivided in classes IIA and IIB as the lethality in the former class was due to a mutant *sup3* background (*sup3-UGA, rX*) and haplo-vital prototrophic derivatives, amenable to testing, could be obtained on replacement of *sup3-UGA, rX* by *sup3*⁺. The lethality in the latter class could not be removed by this procedure. Crosses between the revertants and the five testers were performed and haploid progeny spores plated on yeast extract agar. If the progeny colonies of a particular test cross were only white, it was concluded that the strain under study and the tester carried the same determinant responsible for prototrophy (the same suppressors in the case of suppressor testers, an *ade*⁺ gene in the case of the wild-type tester). This positive indication of allelism was complemented by analysing at least one of the four remaining crosses with the same revertant. Apart from the special situation described below, such crosses gave white and red colonies, the latter being suppressor free *ade6-704* recombinants. In the case of revertants originating from selfings involving *ade1-40* (nos 8–12) progeny from crosses with the wild-type tester had to be replica plated onto minimal agar to check for auxotrophic recombinants since the red pigment marker (*ade6-704*) was absent in both strains of such crosses.

a 2n	h ⁺	ade6-704	sup3-UGA, r36	sup12-UGA
	h ⁻	ade6-704	sup3-UGA, r36	±
b 2n	h ⁺	ade6-704	±	sup12-UGA
	h ⁻	ade6-704	±	±
c n	h ⁻	ade6-704	sup3-UCG	sup12-UGA
d nxn	h ⁺	ade6-704	±	±
	h ⁻	ade6-704	sup3-UCG	sup12-UGA
e n	h ⁻	ade6-704	sup3-UCG	±
f 2nxn	h ⁺	ade6-704	±	sup12-UGA
	h ⁺	ade6-704	±	±
	h ⁻	ade6-704	sup3-UCG	±
g 2nxn	h ⁺	ade6-704	sup3-UGA, r36	sup12-UGA
	h ⁺	ade6-704	sup3-UGA, r36	±
	h ⁻	ade6-704	sup3-UCG	±
h nxn	h ⁺	ade6-704	sup3-UCG	sup12-UGA
	h ⁻	ade6-704	sup3-UCG	sup12-UGA

Fig. 3 Isolation of haplo-vital *sup12-UGA* strains by the generation of a *sup3-UCG* allele. The crosses involved and the strains used are identified with letters a-h. The ploidy of the strains is indicated by n (haploid) and 2n (diploid). For f, g and h crosses with the reciprocal mating-type configuration were also performed, depending on the nature of the isolate to be tested. For further explanations see text.

mental selfings was due to an active suppressor allelic with the doubly-marked input allele in the vast majority of cases (that is, *sup3-UGA, rX* strains produced predominantly *sup3-UGA* revertants and *sup9-UGA, rX* strains produced mostly *sup9-UGA* prototrophs). This contrasts with the controls which gave back mutations at the *ade* loci and forward mutations at the suppressor loci with varying but comparable frequencies. The revertants designated 'others' in Table 1 have not been analysed further. They might comprise weak omnipotent suppressors or allosuppressors²⁵.

On the basis of mutation alone one would have to postulate that the inactivating *r* sites would have an unusually high probability of being changed back to the original wild-type state, thus restoring suppressor activity. This explanation is very unlikely. We therefore rather assume that recombination is responsible for the observed phenomenon. As identical alleles of the marked suppressor gene are involved in selfings, no recombinants are expected to occur following their interaction. We postulate that non-allelic but related wild-type genes (that is, other tRNA^{Ser} genes) interact with the doubly marked gene. According to this interpretation, gene segments carrying the inactivating *r* sites but not the anticodon sequence are replaced by the corresponding sequences of the wild-type partners in a unidirectional process of gene conversion¹⁴.

Besides the observed import of information into the marked suppressor gene, export of the suppressor active anticodon sequence into related wild-type genes is also expected to occur. Prime candidates to act as acceptors are the *sup9*⁺ gene in *sup3-UGA, rX* selfings and the *sup3*⁺ gene in *sup9-UGA, rX* selfings. If such transfers occur non-reciprocally, however, the haploid progeny will be lethal because both tRNA^{Ser} genes will be of mutant constitution, thus depriving the cell of functional tRNA^{Ser} (for example, *sup3-UGA, rX sup9-UGA*)²⁰. That no *sup9-UGA* revertants are observed in selfings involving *sup3-UGA, rX* and vice versa is thus expected and rests on the special situation in *S. pombe* that *sup3*⁺ and *sup9*⁺ are the only two genes producing functional tRNA^{Ser}. The experimental system has therefore to be modified so as to demonstrate export of suppressor active anticodon information into companion wild-type genes.

Export of information generates a novel haplo-lethal suppressor

To determine whether the postulated export of suppressor active anticodon information does occur, we analysed selfings of diploid strains. The results are given in the lower part of Table 1 (expts 13-15) and in Fig. 1. Our interpretation is outlined in Fig. 2. Again, as in the case of haploid strains, the reversion frequency was clearly elevated in selfings involving inactivated suppressor genes as compared with the control. The selfing involving the *sup3-UGA, r36* allele is particularly instructive. Not only did we find prototrophic diploids carrying active *sup3-UGA* alleles, but also strains carrying *sup9-UGA* or a new suppressor, *sup12-UGA*, in significant excess over the control (Fig. 1).

The *sup3-UGA* bearers are explained as in the case of haploid selfings. The increased occurrence of *sup9-UGA* events is taken as evidence for genetic export of the suppressor active anticodon sequence from *sup3-UGA, rX* to *sup9*⁺, again via gene conversion. Note that in this case the two interacting partners are located on different chromosomes (Fig. 2). These strains can survive as diploids, in contrast to the haploid situation, because they harbour one unmutated tRNA^{Ser} gene (*sup9*⁺).

Evidently, a second gene can accept suppressor active anticodon information by the same mechanism, namely *sup12*⁺ (UCG). The original constitution of these revertants is depicted in Fig. 2c. On sporulation, each tetrad gave two lethal and two auxotrophic spores. Thus, *sup12-UGA* is a recessive lethal suppressor. That it is a suppressor in its own right and not an allosuppressor acting in conjunction with *sup3-UGA, rX* was demonstrated by replacing the doubly-marked *sup3* background (for example, *sup3-UGA, r36*) by a homozygous wild-type background (*sup3*⁺). Such diploids of constitution *h*⁺ *ade6-704 sup12-UGA/h*⁻ *ade6-704 sup12*⁺ are still prototrophic and produce two lethal and two auxotrophic segregants per tetrad on sporulation. In addition, *sup12* does not show genetic linkage with either *sup3* or *sup9* (data not shown).

The *sup12* gene, which had not been identified genetically before, is identical with the tRNA^{Ser} gene whose sequence has been published²³ (see below). As *sup3*⁺ and the tRNA^{Ser} gene differ only by two base pairs in their coding regions, it is likely that they can interact as discussed above. Because *sup12-UGA* is haplo-lethal, we conclude that no substitute exists for the product of the tRNA^{Ser} gene. In addition, it is interesting that the change of *sup12*⁺ (UCG) to *sup12-UGA* requires 2 bp changes in the anticodon sequence. This should easily occur via gene conversion but only very rarely by spontaneous mutation.

Haplo-vital *sup12-UGA* strains can be derived by further intergenic exchanges

As genetic analysis with a haplo-lethal suppressor is tedious, we sought a way to change one of the genes coding for UCA reading tRNA to an allele producing a UCG reading tRNA. The procedure to obtain haplo-vital *sup12-UGA* isolates is outlined in Fig. 3. The experiment was started with one of the original *sup12-UGA* diploids derived from selfing 15 (Table 1) and is depicted in Fig. 3a. A derivative of (a) homozygous for one of the mating-type alleles was crossed to an *ade6-704/ade6-704* diploid homozygous for the opposite mating-type allele. From this cross segregant (b) was obtained. On sporulation, vital progeny were dark red exclusively (*ade6-704 sup3*⁺), in contrast to the meiotic progeny of (a) which are all pink, the particular *sup3* allele involved being responsible for this colour difference (*ade6-704 sup3-UGA, r36*). The vast majority of haploid spores from (b) will not grow on minimal agar: 50% are lethal due to *sup12-UGA*, 50% are adenine dependent. Nevertheless, two types of haploid prototrophs might rarely occur via mutation or information transfer: (1)

Active suppressors at the *sup3* or *sup9* locus, if they inherit at the same time *sup12*⁺. They are normal suppressors and will not generate lethal progeny in crosses with standard partner strains. (2) Strains with a mutant anticodon information at *sup3* or *sup9* resulting in the production of tRNA^{Ser}_{UCG} rather than tRNA^{Ser}_{UCA} (for example, strain *c*, Fig. 3). If they carry *sup12*-*UGA* at the same time, such strains are haplo-vital prototrophs; lethality resulting from mutation of the only naturally occurring tRNA^{Ser}_{UCG} producer (*sup12*) has now been by-passed. For instance, *sup3*-*UCG* will produce tRNA^{Ser}_{UCG}, *sup9*⁺ will form tRNA^{Ser}_{UCA}, and *sup12*-*UGA* suppressor-active tRNA^{Ser}_{UGA}. In crosses with standard partners, lethal progeny are expected to be produced due to the separation of *sup3*-*UCG* from *sup12*-*UGA*.

Among $\sim 3 \times 10^7$ spores of strain (*b*), 11 haplo-vital prototrophs were obtained. Ten of them did not segregate lethal progeny in crosses with a standard partner and were discarded. The remaining one of presumed constitution (*c*) was crossed to *h*⁺ *ade6-704* (*d*). The following tetrads were observed: 9 parental ditypes (2 red and 2 white colonies per tetrad), 17 tetratypes (2 red, 1 white, 1 lethal) and 5 non-parental ditypes (2 red and 2 lethal). The same strain (*c*) was also crossed to *h*⁺ *ade6-704 sup3-UGA* and *h*⁺ *ade6-704 sup9-UGA*. These crosses gave results compatible with two and three independent factors segregating, respectively, thus indicating that the derived strain carries a *sup3*-*UCG* rather than a *sup9*-*UCG* allele, as shown in (*c*). Physical evidence that the *sup3*-*UCG* allele originated from intergenic conversion and not from mutation is given below.

Red strains from non-parental ditype tetrads of cross (*d*) should be of constitution (*e*). Although phenotypically not distinguishable from normal red *ade6-704* strains, they are predicted to carry the mutant *sup3* allele *sup3*-*UCG*. This 'helper' strain was crossed to mating-type homozygous derivatives of all 22 *sup12*-*UGA* diploids obtained from selfing 15 (Table 1). These triploid crosses are shown diagrammatically in (*f*) and (*g*). Some of the diploid parents used carried the original *sup3*-*UGA*, *r36* background (*g*), others the *sup3*⁺ background (*f*). In all cases haploid prototrophic recombinants were easily obtained. In contrast, this did not occur if a normal

ade6-704 sup3⁺ strain was the haploid parent in such triploid crosses. All these haploid prototrophs were involved in crosses with each other (cross type (*h*)). In all cases the vast majority of progeny colonies was white. This indicates that the determinants responsible for prototrophy in the original diploids are indeed allelic (*sup12*-*UGA*).

Having obtained haplo-vital *sup12*-*UGA* strains, we proceeded to determine the chromosomal location of this suppressor (data not shown). Based on the analysis of 94 tetrads of a cross homozygous for *ade6-704* and *sup3*-*UCG* and heterozygous for *ura1*, *pro1* and *sup12*-*UGA*, we inferred the arrangement *ura1*-23 centimorgans-*pro1*-9cM-*sup12* on chromosome I²⁶.

Physical evidence for intergenic conversion

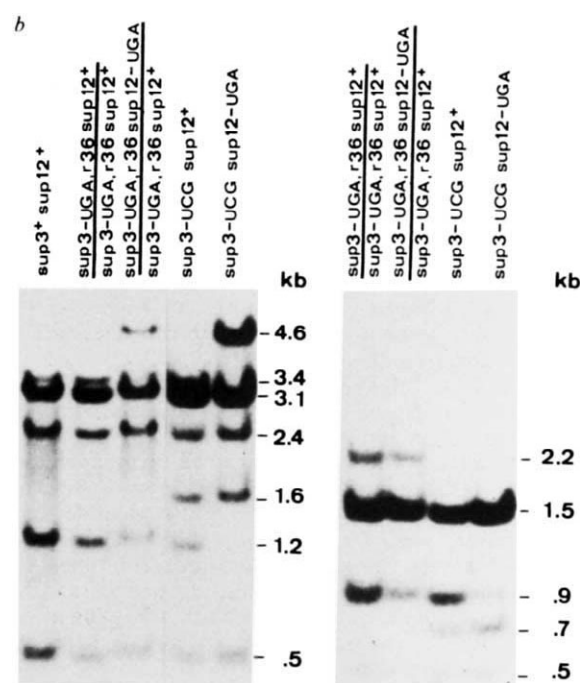
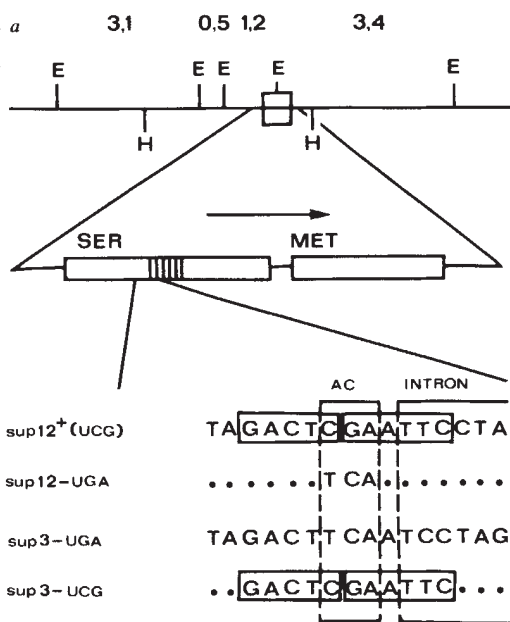
By restriction analysis of DNA from the relevant strains and by filter hybridization experiments according to Southern²⁷ we obtained evidence for the identity of the *sup12*-*UGA* suppressor allele with the known tRNA^{Ser}_{UCG} gene. The data also indicate that non-adjacent base pairs have been substituted in *sup3*-*UCG* in comparison with *sup3*⁺ (Fig. 4). In addition, *sup3*-*UCG* has been sequenced (Fig. 5).

Genomic blots give complex hybridization patterns, because one is dealing with a family of cross-hybridizing genes. In addition to the well characterized *sup3*, *sup9* and *sup12* genes there is a further related sequence present in the genome (data not shown). Figure 4a gives a simplified restriction map of the chromosomal region with the tRNA^{Ser}_{UCG} gene and the sequence of its non-coding strand comprising the anticodon and the 5' part of the intron²³. The sequence of the corresponding region of *sup3*-*UGA* is also given²¹.

The phenotypic effect of *sup12*-*UGA* concerning suppression is identical with that of the other opal suppressors inserting serine (*sup3*-*UGA*) or leucine (*sup8*-*UGA*) which are known to code for suppressor tRNAs with the anticodon U*CA^{28,29}. Thus, *sup12*-*UGA* most probably carries the anticodon sequence TCA which abolishes the two restriction sites present at this location in *sup12*⁺ (Fig. 4a). In fact, a new and longer *Eco*RI restriction fragment is found in DNA of *sup12*-*UGA*/*sup12*⁺ diploids. In the haplo-vital *sup3*-*UCG* *sup12*-

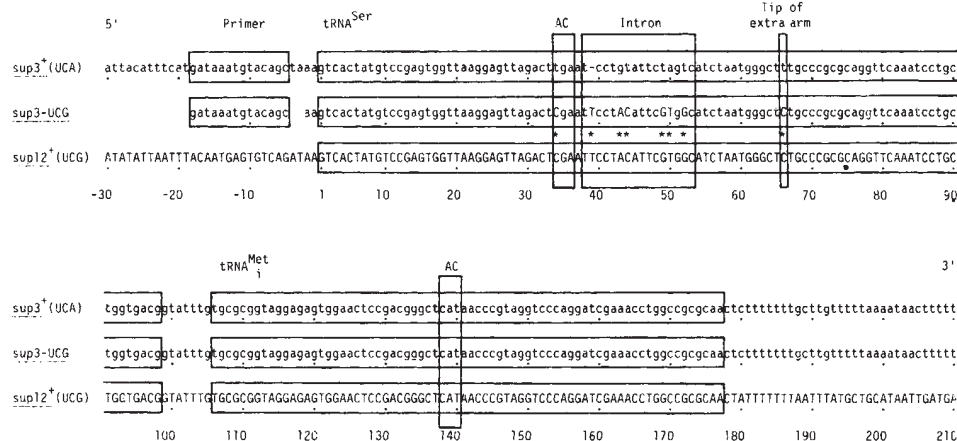
Fig. 4 Physical evidence for *a*

intergenic conversion. *a*, The chromosomal organization of the *sup12* locus and the relevant sequences of the anticodon regions of *sup12*⁺ and *sup3*-*UGA* are represented^{21,23}. The *Eco*RI restriction sites (E) and the size of the resulting fragments in kilobases are indicated. The two *Hind*III sites (H) delimit the cloned fragment present in the chimaeric plasmid pYM118 that carries the tRNA^{Ser}_{UCG}-tRNA^{Met} genes²³. In the blow up the tRNA genes are shown as boxes. The shaded area signifies the intron and the arrow the direction of transcription. Below, excerpts of the published sequences of *sup12*⁺ (ref. 23) and *sup3*-*UGA*²¹ are given as well as the deduced sequences of the two intergenic convertants analysed. The anticodons (AC) and the 5' border of the introns are marked with broken lines. The relevant restriction sites recognized by the *Eco*RI (GAATTC) and *Hinf*I (GANTC) endonucleases are boxed.



b, On the left hybridization patterns according to Southern²⁷ are shown that were obtained after digestion of genomic DNA with *Eco*RI. The corresponding results obtained with *Hinf*I digests are given on the right. The hybridization probe consisted in the nick-translated *Hind*III fragment from the recombinant plasmid pYM118²³. Above each lane the genotype of the haploid or diploid strain is given that served as source of the DNA. The novel *Eco*RI fragment of 4.6 kb length is present in strains carrying *sup12*-*UGA*, while the new fragment of 1.6 kb length correlates with *sup3*-*UCG*. In strains with the *sup3*-*UCG* allele the 2.2 kb *Hinf*I fragment is missing and a new 0.7 kb fragment appears. For more details see the text.

Fig. 5 Nucleotide sequence of the intergenic convertant *sup3-UGG*. The non-coding strands of the tRNA genes *sup3⁺* (small letters) and *sup12⁺* (capitals) and their flanking sequences were taken from refs 21 and 23. Between them the sequence of *sup3-UGG* is given. It differs from *sup3⁺* at all of the eight positions (capitals) that vary between *sup3⁺* and *sup12⁺* (asterisks). Thus, the conversion event includes a block of at least 33 nucleotides. No change was observed between *sup3-UGG* and *sup3⁺* in the 3'-flanking region for the first 40 nucleotides downstream of the tRNA^{Met} gene. In the 5'-flanking region at least the primer oligonucleotide sequence must be conserved. DNA from a strain carrying *sup3-UGG* was digested with *Hind*III and ligated into the plasmid pTR262 (ref. 32) which allows positive selection for chimaeric plasmids. Plasmids harbouring *sup3-UGG* were identified by colony hybridization with a nick-translated probe carrying the *sup3-UGA* gene. Subsequently, a *Hind*III–*Bam*HI fragment of 950 bp carrying the *sup3-UGG* gene was transferred from the chimaeric plasmid to the phage M13mp8 (ref. 33). Single-stranded phage DNA served as template for nucleotide sequencing according to the dideoxy termination method³⁴ using as primer a synthetic tetradecamer; its nucleotide sequence is boxed. The primer (Applied Biosystems) was a gift of Dr D. Söhl. Detailed cloning and sequencing procedures will be described elsewhere.



UGA strain the new 4.6-kb fragment is still visible, while its two constituting subfragments of 3.4 and 1.2 kb length (as present in *sup12⁺* DNA) are missing (Fig. 4b). Surprisingly, no longer fragment appears in the corresponding *Hinf*I digests. When the sequences in the more distal parts of the introns of *sup12⁺* and *sup3-UGA* (Fig. 5) are examined, one discovers that there is the potential for the creation of a new *Hinf*I site in *sup12-UGA* by conversion. This explanation for the lack of a larger *Hinf*I fragment in *sup12-UGA* strains has to be tested by nucleotide sequencing.

The *sup3-UGG* convertant (presumed anticodon CGA) was derived from *sup3⁺* (anticodon TGA) as described above. But it has been shown that *sup3-UGG* convertants can also be obtained starting with *sup3-UGA* alleles (R. Aebi, C. Gysler & W. Heyer, personal communication). A new *Eco*RI site should be created in the *sup3-UGG* allele only if the convertant received its anticodon sequence from *sup12⁺* (anticodon CGA) together with at least part of the intron of *sup12⁺* (see Fig. 4a). In fact, the strains carrying the *sup3-UGG* allele yield a new shorter *Eco*RI fragment of 1.6 kb length (Fig. 4b). If a homologous *sup3* hybridization probe is used instead of the heterologous *sup12* probe an additional new fragment of 0.8 kb appears (data not shown). A band corresponding to the original *sup3* fragment of 2.4 kb (1.6 + 0.8 kb) is always visible, because it coincides with an *Eco*RI fragment from one of the other cross-hybridizing genes. Finally, the presence of the *Hinf*I site in the *sup3-UGG* allele (in contrast to *sup3-UGA*) is documented by the disappearance of the 2.2 kb *Hinf*I fragment in DNA from *sup3-UGG* strains (Fig. 4b). Instead, a new fragment of 0.7 kb length hybridizes to the probe, whereas the other subfragment of 1.5 kb length coincides with a fragment that stems from the *sup12* gene.

Our interpretation of these and further data not shown here is that multiple and nonadjacent base-pair changes occurred during the creation of the particular *sup12-UGA* and *sup3-UGG* alleles. This is best explained by intergenic conversion. To confirm this interpretation we are now cloning and sequencing the relevant genes. Very recent nucleotide sequence data concerning *sup3-UGG* actually show that in this intergenic convertant the whole intron sequence (differing at six nonadjacent positions from the *sup3⁺* intron sequence) and the sequence coding for the loop of the extra arm (differing at one position from *sup3⁺*) have been transferred from *sup12* to *sup3* (Fig. 5).

Discussion

From the evaluation of selfings of auxotrophic nonsense mutants carrying inactivated suppressors we conclude that re-combinational information transfer occurs between the three unlinked serine tRNA genes *sup3*, *sup12* (both on chromosome

I) and *sup9* (chromosome III) in *S. pombe*. This interpretation, in contrast to mutation, is discussed in detail in an earlier publication¹⁹. The crucial points are: (1) Much more prototrophs are obtained in selfings of inactivated suppressors (frequency range: 1.4×10^{-7} to 20×10^{-7}) than in controls that measure the mutational background (0.3×10^{-7} , Table 1). (2) In selfings of haploids the large majority of prototrophs (> 90%) are due to import of wild-type information into the marked tRNA gene. The gene thereby loses the inactivating secondary lesion and becomes suppressor active again. Only a minority of prototrophs (<10%, Table 1) are due to mutational events elsewhere in the genome. In selfings of diploid strains which permit to detect even recessive lethal events, we have now demonstrated in addition export of suppressor active anticodon information from the inactivated gene to other genes of the family. In this way a new opal suppressor has been obtained spontaneously that was never observed in controls (frequency: 1.7×10^{-7} in selfings of the inactivated allele *sup3-UGA*, r36). Restriction analysis and nucleotide sequencing has revealed multiple and nonadjacent base-pair changes in specific convertants.

The mechanisms responsible for information transfer between unlinked genes may be identical or related to the ones that operate in homologous recombination between alleles¹⁴. If this is true, it follows from the present results that blocks of conserved sequences as short as 200 bp embedded in unrelated sequences are sufficient for the initiation of hybrid-DNA formation, which with or without subsequent mismatch repair can result in gene conversion. This interaction may take place between short conserved sequences in the lateral DNA loops of different chromosomes.

Recently it has been shown *in vitro* that *recA* protein from *E. coli* is unable to extend hybrid-DNA formation through regions of DNA with an average of 1 mismatch per 10 bp³⁰. Our own results show that eight mismatches within a stretch of 20 bp are no obstacle to exchanges between *sup3* and *sup12* *in vivo*. It is true that suppressor anticodon export from *sup3-UGA*, rX to *sup12⁺* is only half as frequent as export to *sup9⁺* that carries an intron identical to *sup3* (Fig. 1). But there may be alternative explanations for this discrepancy, for example, effects due to the specific chromosomal location of the genes involved.

If events of intergenic conversion were frequently associated with crossing-over as in inter-allelic gene conversion, various chromosomal rearrangements would result and many such events would be lethal. In a first search no chromosomal rearrangements were identified among intergenic convertants (C. Gysler, personal communication). However, more has to be known about the orientation of the three tRNA genes with

respect to their centromeres before the potential for the creation of viable rearrangements can be assessed.

More light may be shed on the mechanisms of intergenic conversion by studying other tRNA gene families. Experiments with suppressors derived from two leucine tRNA genes (not shown) indicate that they participate less frequently in intergenic conversion events. This may be due to shorter blocks of near-identical sequence in this gene family, since as a rule tRNA genes are not arranged in multimers in eukaryotes. This results in blocks of conserved sequence of not more than 100 bp.

Like inter-allelic recombination, intergenic conversion also seems to be influenced by specific marker effects. In the serine tRNA genes certain second-site mutations display vastly enhanced recombination frequencies in inter-allelic recombination²⁴. The one such second-site mutant tested, *sup3-UGA,r10*, gives also the highest frequency of intergenic conversion (Table 1).

It has been shown experimentally that intergenic conversion occurs in various other systems¹⁵⁻¹⁸ (see introduction). One additional example of *in vivo* formation of a 'hybrid' tRNA gene derived from two different tRNA genes has been discovered in the bacteriophage T4 (ref. 31 and W. McClain, personal communication). Intergenic conversion is likely to be the main mechanism responsible for concerted evolution of

families of dispersed genes and also of linked genes^{6,7,16,17}. It may propagate or eliminate specific mutations within a gene family and in this way contribute to conservative evolution and rapid sequence change. A recent review on the evolution of tRNA sequences includes a thorough discussion of the problems encountered in the construction of phylogenetic trees based on sequence comparisons³¹. Here we stress that the occurrence of intergenic conversion violates one of the fundamental assumptions used in the construction of phylogenetic trees, namely that the daughter copies will not exchange sequences after a gene has been duplicated.

The described selection of serine tRNA gene variants which are altered in intron sequences but remain functional at the tRNA level will prove useful for an understanding of tRNA biosynthesis (removal of intron sequences). From sequence data on inactive tRNA gene variants which carry secondary lesions introduced by either mutation or intergenic conversion one will also learn more about tRNA function.

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Identification of the class I genes of the mouse major histocompatibility complex by DNA-mediated gene transfer

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DNA-mediated gene transfer was used to identify cloned class I genes from the major histocompatibility complex of the BALB/c mouse. Three genes encoding the transplantation antigens H-2 K^d, D^d and L^d were identified as well as genes encoding the Qa-2,3 and two TL differentiation antigens. As many as 10 putative novel class I genes were detected by the association of their gene products with β_2 -microglobulin. Alloantiserum prepared to one of the novel antigens was used to demonstrate the expression of the previously undetected antigen on spleen cells of various inbred, congenic, and recombinant congenic strains of mice.

THE major histocompatibility complex of the mouse encodes a family of cell-surface antigens known as the class I molecules. These gene products, which fall into two categories, are encoded by distinct regions of the major histocompatibility complex. The H-2 region encodes the transplantation antigens which were initially defined by allograft rejection^{1,2}. These antigens are expressed on most somatic cells of the mouse and are

involved in the T-cell recognition of cells altered by viral or neoplastic transformation (for a review see refs 3,4). The second category of class I antigens are the haematopoietic differentiation antigens encoded by genes in the Tla region. The Tla region encodes three distinct types of differentiation antigens, Qa-1, Qa-2,3 and TL⁵. The TL antigens are expressed only on certain subpopulations of thymocytes and their

Table 1 Summary of the coding assignments for the class I genomic clones

Clone	Reaction of transformants with antibodies specific for					Assignment
	K ^d	D ^d	L ^d	Qa-2,3	TL ^c	
λ1.3	+					K ^d
λ26.1	+					K ^d
λ21.1	+					K ^d
c17.1	+					K ^d
c18.1		+				D ^d
λ27.5			+			L ^d
c59.2			+			L ^d
c50.2				+		Qa-2,3
λ17.3					+	TL
c6.3					+	TL
λ24.8					+	TL
66.1					+	TL

Mouse L tk cells were co-transformed with ~1 µg of DNA from each of the λ and cosmid clones (see text) and the HSV tk gene in pBR322 (ptk5) as previously described¹⁸. For those cosmid clones containing more than one gene, the clone DNA was digested with an appropriate restriction enzyme to inactivate all but a single gene for transformation (see ref. 24). Uncloned tk⁺ transformants selected in hypoxanthine/aminopterin/thymidine medium³⁸ were panel analysed by radioimmunoassay as previously described¹⁸ using monoclonal hybridoma antibodies 34-1-2 to K^d (ref. 39), 34-2-12 to D^d (ref. 39), 30-5-7 to L^d (ref. 40), D3.262 to Qa-2,3 (ref. 41) and TLm(i) to TL³³. Positives are indicated for those transformants which yielded at least 5,000 c.p.m. of iodinated (¹²⁵I) protein A bound (see Figs 2, 4) with a 10⁻² or 10⁻³ dilution of the ascites fluid in duplicate transformation experiments. Background for the various antibodies and transformants ranged over 100 to 300 c.p.m., except for the IgM Qa antibodies which utilized a facilitating reagent (see Fig. 4). The active genes on cosmid clone 17.1, 18.1, 59.2, 50.2, 6.3 and 66.2 are the first gene on cluster 11, the first gene on cluster 13, the second gene on cluster 2, the second gene on cluster 6, the second gene on cluster 4, and the first gene on cluster 5, respectively (see Fig. 5).

neoplastic counterparts in certain T-cell leukaemias⁶. The Qa-1 antigen is expressed by thymocytes, peripheral T and B cells, as well as lymphoblasts⁷. The Qa-2,3 antigens are found on a subset of bone marrow-derived cells⁸. The biological functions of the TL or Qa antigens are not known.

Class I antigens have been characterized using specific alloantisera and monoclonal antibodies prepared by the appropriate cross-immunizations with cells from inbred, congenic and recombinant strains of mice. Serological analysis has demonstrated that the transplantation antigens are extremely polymorphic and that different strains of mice have distinct combinations or haplotypes of class I alleles. Particular class I molecules are denoted by appropriate letters with a superscript small letter for the haplotype. For example, the serologically defined transplantation antigens of the BALB/c mouse which is of the H-2^d haplotype are denoted K^d, D^d, L^d or R^d.

Class I molecules of both categories appear to share a common structure. The class I polypeptide is a 45,000-molecular weight integral membrane protein which is noncovalently associated with a 12,000-molecular weight polypeptide, β₂-microglobulin. Class I molecules consist of three external domains, each of ~90 amino acids, a transmembrane region of ~40 residues, and one cytoplasmic domain of 30 residues^{9,10}.

Class I cDNA clones have been characterized and used as probes to obtain genomic clones containing class I genes¹¹⁻¹⁷. Two genes isolated from a BALB/c Crgl sperm DNA library constructed with the lambda bacteriophage (λ) vector have been extensively characterized. The class I gene from clone λ27.1 is a pseudogene which has been mapped to the Qa-2,3 subregion of the Tla region by restriction enzyme site polymorphisms¹⁵. DNA sequence analysis of gene 27.1 shows that this class I gene is divided into eight distinct exons encoding each of the individual domains of a class I molecule. Recently, DNA-mediated gene transfer and radioimmunoassays were used to demonstrate that a gene from clone λ27.5 encodes the H-2L^d transplantation molecule¹⁸. The L^d molecules produced by mouse L cells transformed with λ27.5 DNA were shown to be virtually indistinguishable from the L^d molecules expressed on spleen cells from BALB/c mice by biochemical, immunological and functional criteria¹⁸⁻²¹. The DNA sequence of gene 27.5 is identical to that of the L^d antigen at all 77 positions that can be compared²². Other laboratories have also used gene transfer to identify the cloned class I genes from libraries constructed from tissues or tumour cell lines from other sub-strains¹⁷ and strains²³ of mice.

In an attempt to assess the total number of class I genes contained in the BALB/c mouse, genomic clones containing class I genes have been isolated from a cosmid library constructed from BALB/c sperm DNA²⁴. Fifty-four cosmid clones containing 36 unique class I genes have been assigned to 13 distinct groups or clusters encompassing 837 kilobases of DNA. These include most of the class I genes in the BALB/c mouse.

Here, we have used gene transfer and serological analyses to examine individual class I genomic clones from the λ and cosmid libraries. Cloned class I genes encoding the serologically defined H-2 K^d, D^d, L^d, Qa-2,3 and TL antigens have been identified. Evidence is also presented for as many as 10 additional novel class I genes encoding products not previously defined serologically.

Analysis of 96 λ and 36 cosmid class I clones by gene transfer

In an attempt to examine most class I genes from the BALB/c mouse, 96 genomic clones isolated from the λ library and 36 genomic clones previously isolated from the cosmid library (designated c) were analysed. DNA from each of these genomic clones, together with the herpes viral thymidine kinase gene, was used to co-transform thymidine kinase-negative (tk⁻) mouse L cells as previously described¹⁸. The class I H-2^d

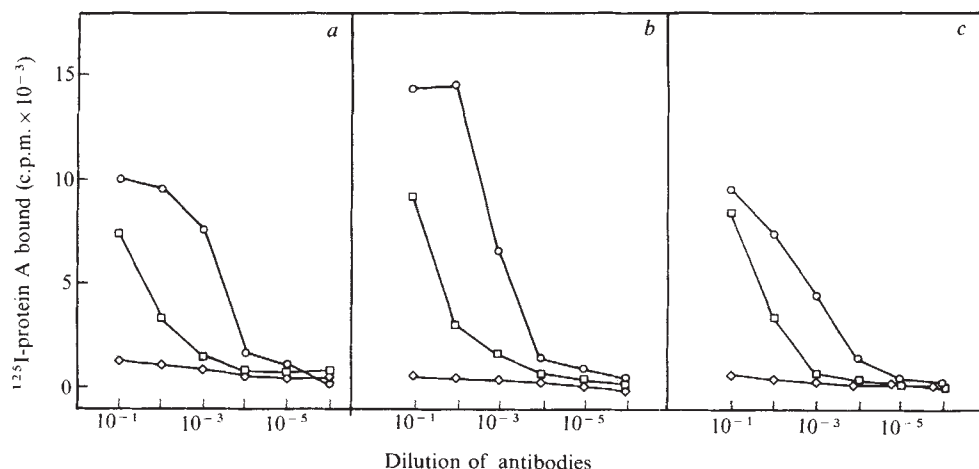


Fig. 1 Radioimmunoassay of λ1.3, λ21.1 and λ26.1 transformants. Transformants which reacted in the panel analysis (Table 1) with antibodies 34-1-2 to K^d molecules were tested by the cell-binding radioimmunoassay with: ○, 34-1-2 antibodies to K^d; □, 20-8-4 antibodies to K^b which react with K^d molecules; and ◇, 34-2-12 antibodies to D^d. Transformants derived from transfection with DNA from clones λ26.1 (a), λ21.1 (b) and λ1.3 (c). Spleen and Ltk⁺ controls are shown in Fig. 4.

products of the transformed L cells were detected by radioimmunoassay and analysed by two-dimensional gel electrophoresis. Since mouse L cells are fibroblasts derived from C3H mice of the $H-2^k$ haplotype, the endogenous K^k and D^k transplantation antigens can readily be distinguished from any of the BALB/c class I molecules of the $H-2^d$ haplotype by appropriate monoclonal antibodies. The mouse L-cell fibroblasts do not express the differentiation antigens Qa-1, Qa-2,3 or TL. These results are summarized in Table 1. In almost all cases, the binding of specific antibodies to the transformants was comparable to that of the binding of the antibodies to the corresponding antigens and not cross-reacting antigens (see Fig. 4).

Identification of genes encoding the H-2 K, D and L transplantation antigens

The L-cell transformants derived from transfections with the $\lambda 1.3$, $\lambda 26.1$ and $\lambda 21.1$ clones express K^d molecules which are virtually indistinguishable in their patterns of serological reactivity with two different monoclonal antibodies to the K^d molecule (Fig. 1), both of which react as strongly with these transformants as they do with spleen cells (see Fig. 4). No reactivity is observed with these four transformants using monoclonal antibodies specific for D^d or L^d antigens (see Fig. 1 legend). Antibodies specific for the K^d molecule do not react with mouse L cells transformed only with the thymidine kinase gene (see Fig. 4). These are designated tk^+ transformants. To determine whether these three class I genes encode distinct gene products, the antigens were isolated by immunoprecipitation and analysed by two-dimensional gel electrophoresis (Fig. 2). As the class I antigens of different haplotypes each have distinct constellations of spots²⁵, we believe this approach is very discriminating. The products of class I genes 1.3, 26.1 and 21.1 appear extremely similar to one another and to the K^d molecules isolated from normal BALB/c spleen lymphocytes. However, minor electrophoretic differences were observed in the faint, lower molecular weight species (Fig. 2a and 2e).

As the electrophoretic patterns between products obtained from duplicate transformations appear identical, the variation in the lower molecular weight products may reflect differences in post-translational modifications of these molecules or differential alternative splicing, as has been previously suggested for class I genes¹⁵. Restriction map analyses of clones $\lambda 1.3$, $\lambda 26.1$ and $\lambda 21.1$ (Fig. 3), however, suggest that the K^d genes they contain are identical to one another and also to the first gene on cluster 11 or clone c17.1 (see Fig. 5) which contains a K^d gene by transformation (Table 1, Fig. 4a). Thus, all of these clones appear to encode very similar or identical genes on an independently derived series of what we believe are overlapping clones, although we cannot exclude the possibility that certain of these genes may actually be distinct and yet appear identical by the limited restriction mapping we have carried out to date. These genes are currently being sequenced in order to resolve this issue.

Cosmid clone 18.1 contains an H-2D^d gene as demonstrated by the radioimmunoassay of the c18.1 transformants with monoclonal antibodies to the D^d antigen (Table 1, Fig. 4b). The 18.1 transformants reacted with antibodies specific for D^d products and not reagents which detect K^d , L^d , TL or Qa antigens. The D^d molecules expressed by the transformants are currently being compared with the molecules isolated from spleen cells by two-dimensional gels and tryptic peptide map analysis to confirm this identification. No lambda clones containing an H-2 D^d gene were identified (see below).

An H-2L^d gene has been identified on cosmid clone 59.2 by transformation (Table 1) and comparison of restriction maps between the c59.2 and $\lambda 27.5$ genes²⁴. The L^d molecules expressed on the corresponding transformants appear indistinguishable in radioimmunoassays from the products of the previously identified gene contained in clone $\lambda 27.5$ (Fig. 4c).

The analyses based on 132 independent transformations resulted in the identification of at least one H-2K^d, a single H-2D^d and one H-2L^d gene. Serological evidence suggests that in some mice there may be multiple K and D gene products²⁶⁻³¹, and additional transplantation antigens such as R^d (ref. 32).

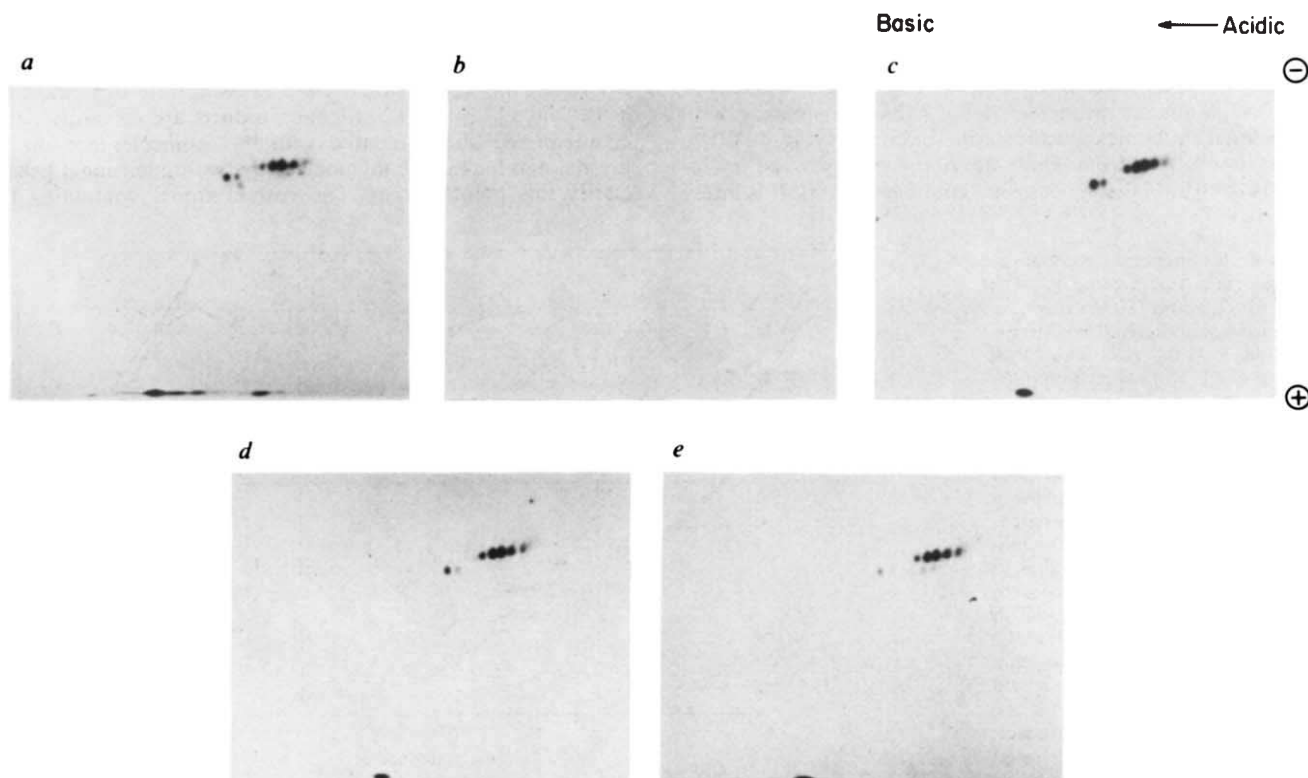


Fig. 2 Fluorographs of two-dimensional gels of H-2K^d molecules immunoprecipitated from cell lysates of BALB/cJ spleen lymphocytes (a), tk^+ cells transformed only with the tk gene (b), $\lambda 1.3$ (c), $\lambda 26.1$ (d) and $\lambda 21.1$ (e) transformants. Cells were biosynthetically radiolabelled, immunoprecipitated with 20-8-4 monoclonal antibodies (supernatant fluids), and analysed by two-dimensional gel electrophoresis as previously described¹⁸. The gels were prepared for fluorography, dried and exposed for ~7 days.

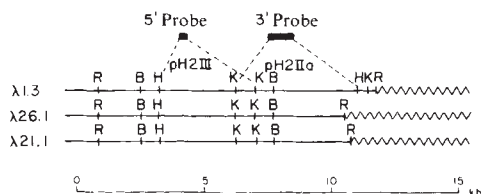


Fig. 3 Restriction maps of the lambda clones containing the K^d genes. DNA from each of the λ clones which yielded K^d -positive transformants was restriction-mapped with the indicated enzymes by single and double digestion as previously described²⁴. The 5' probe pH-2III and the 3' probe pH-2IIa¹¹ were used to detect fragments containing coding sequences homologous to the class I genes (dashed lines). The pH-2III probe contains the sequences encoding amino acids 63–160 of a transplantation antigen and pH-2IIa amino acids 167–352. The coding sequences were orientated using sequencing subclones as previously described²². The solid line indicates the insert DNA, and the λ vector DNA is designated by the wavy lines. R, *EcoRI*; B, *BamHI*; H, *HindIII*; K, *KpnI*. The 3' *EcoRI* sites mark the linkers used in constructing the library.

The failure to find multiple class I K^d and D^d genes may be explained in several ways. First, the serological multiplicity of K or D antigens may arise from post-translational modifications or alternative patterns of RNA splicing. Second, different inbred strains may have different numbers of K and D genes. Third, multiple class I genes might appear identical by restriction mapping and still differ in their DNA sequences. In this case, multiple distinct genes might be confused with a single gene present on overlapping clones. Finally, these genes may not have been detected for various technical reasons, for example, failure to clone the gene or express the gene at levels detectable in the radioimmunoassay. For example, the radioimmunoassay is less effective with heterogeneous alloantisera and specific monoclonal reagents for the R^d or the $Qa-1$ gene products are lacking.

Fibroblast expression of gene encoding the mouse haematopoietic differentiation antigen Qa-2,3

Transformants from c50.2 DNA seem to express low levels of the Qa-2,3 antigen as detected by radioimmunoassay with monoclonal antibodies specific for these products (Fig. 4d). The BALB/c substrain from which the library was derived is Qa-2,3-positive (L. Flaherty, personal communication). It is inter-

esting that Qa-2,3, a gene normally expressed only in haematopoietic cells, appears to be expressed in transformed fibroblasts. The fact that the Qa-2,3 differentiation antigen is expressed at low levels on haematopoietic cells⁸ may explain why limited numbers of Qa-2,3 molecules are expressed in the transformed mouse L cells. It is difficult to ascertain the basis for the low level of expression of this antigen by the transformants. Several possibilities are now being investigated. For example, only rare transformants may have incorporated sufficient copies of the gene to override differentiation signals. In addition, glycosylation of the polypeptide in fibroblasts might, in this particular instance, alter the antigenicity of the molecule causing a less avid reaction with the antibodies. The fact that the c50.2 transformants exhibit elevated levels of β_2 -microglobulin (see below) does suggest that significant amounts of the antigen may be expressed in an altered form associated with β_2 -microglobulin. Accordingly, it has been difficult to obtain sufficient Qa-2,3 antigen from spleen cells or transformed L cells for two-dimensional gel analysis. The identification of a Qa-2,3 gene should readily permit its characterization at the DNA level.

Two different class I genes encode two distinct TL T-cell differentiation antigens

The TL antigens are expressed only on thymocytes at a certain stage of differentiation. In some inbred strains of mice, distinct TL molecules are expressed on certain leukaemic cells of T-cell origin⁶. Two monoclonal antibodies can distinguish these TL antigens. Antibodies denoted TLM(i) recognize TL determinants present on both normal and leukaemic cells, whereas antibodies designated TLM(iii) react only with the TL determinants on normal thymocytes³³. Figure 4e, f shows that transformants from λ clone 17.3 reacted with both monoclonal antibodies at levels comparable to those observed with BALB/c thymocytes (Fig. 4e, f), whereas transformants from λ clone 24.8 reacted only with monoclonal antibodies TLM(i). These observations suggest that there are at least two distinct genes, $\lambda 17.3$ and $\lambda 24.8$, which encode two TL antigens. From the serological analyses described above, gene $\lambda 17.3$ may be expressed on normal BALB/c thymocytes and leukaemic cells whereas gene $\lambda 24.8$ would be expressed only on leukaemic cells. The $\lambda 17.3$ and $\lambda 24.8$ gene products are currently being characterized and compared with the molecules present on normal and leukaemic thymocytes by two-dimensional gels to clarify this point further. The cosmid clones containing the

Fig. 4 Radioimmunoassay of the transformants expressing K^d , D^d , L^d , Qa-2,3 and TL antigens. a, Transformants derived from transfection of L tk⁻ cells with DNA from c17.1 (○) were tested by radioimmunoassay for the expression of K^d molecules using 34-1-2 antibodies to K^d . b, Transformants from transfection with DNA from c18.1 (○) and shown to express D^d molecules as detected in the panel analysis were analysed using 34-2-12 antibodies to D^d . c, Transformants derived from transfection with DNA from $\lambda 27.5$ (○), which contains the L^d gene^{18,22}, and transformants from DNA from c59.2 (●) were tested against 30-5-7 antibodies to L^d . d, Transformants from transfection with c50.2 DNA (○) were tested in a cell-binding radioimmunoassay using D3.262 monoclonal hybridoma IgM antibodies to Qa-2. Rabbit antiserum to mouse IgM (Bionetics) was used as a second step reagent before incubation of the cells with ¹²⁵I-protein A. e, Transformants derived from transfection with DNA from $\lambda 17.3$ were tested against antibodies to TL (●) TLM(i) and (○) TLM(iii) (see text). f, Transformants from transfection with $\lambda 24.8$ DNA were tested against the same antibodies to TL indicated in e. BALB/c spleen cells, panels a–d (□) or thymocytes, panels e–f (□) and Ltk⁺ cells, panels a–f (△) were run as controls. Thymocytes and Ltk⁺ cells in panel e were tested against TLM(i), panel f against TLM(iii).

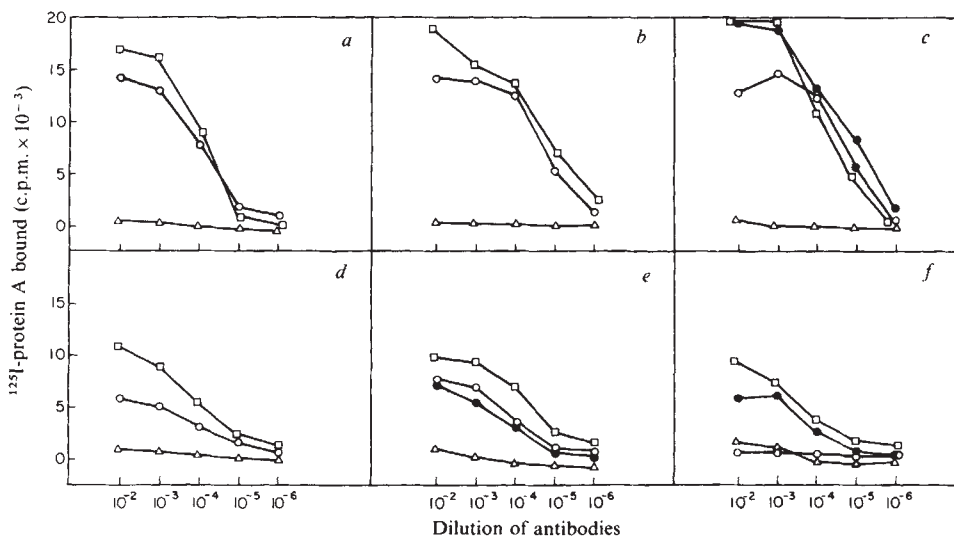
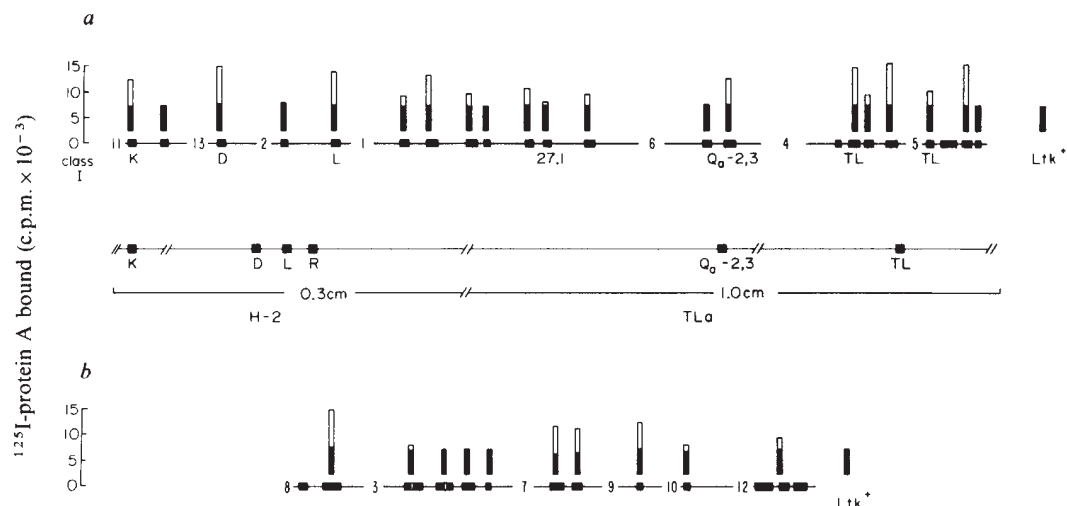


Fig. 5 Radioimmunoassay of cosmid transformants for β_2 -microglobulin. The mixed population of tk^+ transformants derived from transfection with a single gene from each of the various cosmid clones, obtained by restriction digestion, was tested for the expression of β_2 -microglobulin in a cell-binding radioimmunoassay using rabbit anti-serum to rat β_2 -microglobulin. Each transformant was assayed in duplicate against a 10^{-2} dilution of the anti-serum. The numbers



shown at the left indicate the c.p.m. of ^{125}I -protein A bound per 50,000 cells. Less than 10% variation in the number of c.p.m. was observed between duplicate transformations or assays. The dark portion of each bar represents the basal level of L-cell β_2 -microglobulin measured for tk^+ transformants which is the average of duplicate samples tested (right). The open portion of each bar represents the total number of c.p.m. of ^{125}I -protein A bound for each transformant which exceeds the level on tk^+ cells. Cosmid clusters are represented schematically with the genes indicated as the enclosed boxes. Each cluster is comprised of a group of noncontiguous overlapping clones²⁴. The cosmid cluster number is indicated to the left of the horizontal black line and the genes encoding the serologically defined products are indicated below the appropriate boxes. *a*, β_2 -microglobulin on the surface of transformants derived from transfection with DNA from cosmid clones linked with mapped genes or genes encoding serologically defined antigens; *b*, undefined clusters only.

corresponding TL genes have been identified by transformation (Table 1, Fig. 5). The 17.3 TL gene appears to be the first gene on cosmid cluster 5 or clone 6.3, and the 24.8 gene the second gene on cosmid cluster 4 or clone 66.1. Since each TL gene maps to a separate cosmid cluster, they must be distinct genes. These results illustrate two important points regarding gene transfer into mouse L cells. First, as was noted for the Q₀-2,3 gene, the TL genes which are developmentally regulated and expressed only on thymocytes can be expressed in fibroblasts as a result of transformation. Second, genes not expressed in the mouse except on leukaemias, such as gene $\lambda 24.8$, can be expressed by transformed fibroblasts. It seems unlikely that the activation of endogenous L-cell genes encoding similar TL molecules would account for these results as restriction digestion of $\lambda 17.3$ or $\lambda 24.8$ DNA destroys the ability of these genes to transform L cells to the TL-positive phenotype.

Ten class I genes encode serologically undefined gene products

Of the 36 distinct class I clones, six contain genes encoding serologically defined gene products (Fig. 5a). The remaining class I genes are either pseudogenes or encode as yet unidentified cell-surface products. For example, they could encode nonpolymorphic molecules expressed on the surface of most cells which therefore would not have been detected by classical serological approaches. Alternatively, they might be differentiation antigens expressed at defined stages on tissues not yet tested for their ability to generate alloantisera. Cytoplasmic or secreted forms of class I molecules might represent another category of uncharacterized products.

We have used a simple but effective approach to identify class I gene products for which there are no specific serological reagents. All class I molecules studied associate with β_2 -microglobulin before expression on the cell surface^{34,35}. Therefore, the expression of foreign class I molecules on the surface of the mouse L cell should result in the elevation of the amount of β_2 -microglobulin expressed on the cell surface. To test this hypothesis, a radioimmunoassay for cell-surface β_2 -microglobulin was developed and used to quantitate the amount of β_2 -microglobulin on several transformants expressing foreign serologically defined class I molecules as well as several which failed to react with any of the class I-specific antibodies. The tk^+ transformants express a basal level of β_2 -microglobulin as do the mixed population of tk^+ transformants derived from

transfection with the L^d gene cleaved into small coding fragments with *Bam*HI enzyme. In contrast, mouse L cells transformed with the L^d, D^d, K^d and TL genes all expressed levels of cell-surface β_2 -microglobulin well above the background level as measured by radioimmunoassay (Fig. 5a). This increase is reproducible in that the measured levels of β_2 -microglobulin obtained for duplicate assays or transformations vary by less than 10%. In addition, the elevation in β_2 -microglobulin expression appears to be a stable phenotype, observed after 50 passages of some of the cell lines in culture. If elevated β_2 -microglobulin expression results from the expression of additional class I molecules by the transformants, this phenotype would be stably expressed because it has been demonstrated

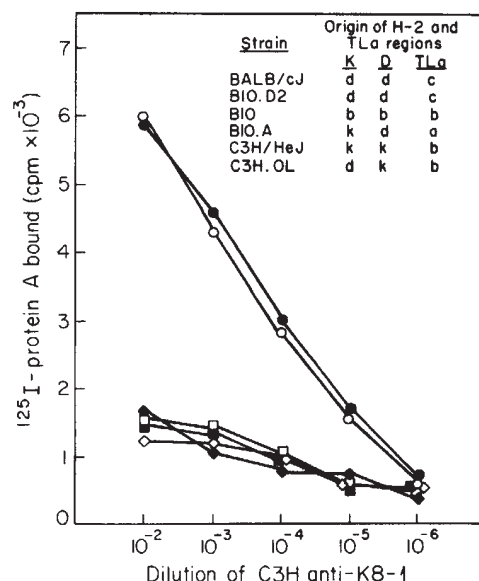


Fig. 6 Radioimmunoassay of spleen cells with C3H antibodies to K8-1 transformants. Four C3H mice were immunized by injecting 5×10^7 UV-irradiated K8-1 cells intraperitoneally every 2 weeks for 8 weeks. Mice were bled 7 days after the last injection and the antiserum used in the cell-binding radioimmunoassay against spleen cells as previously described¹⁸. Reaction of spleen cells from: $\bullet$, BALB/cJ; $\circ$, BIO.D2; $\blacksquare$, C3H/HeJ; $\square$, C3H.OL; $\blacklozenge$, BIO; $\diamond$, BIO.A. The origins of the H-2 and Tla regions for each of the mice are indicated in the panel.

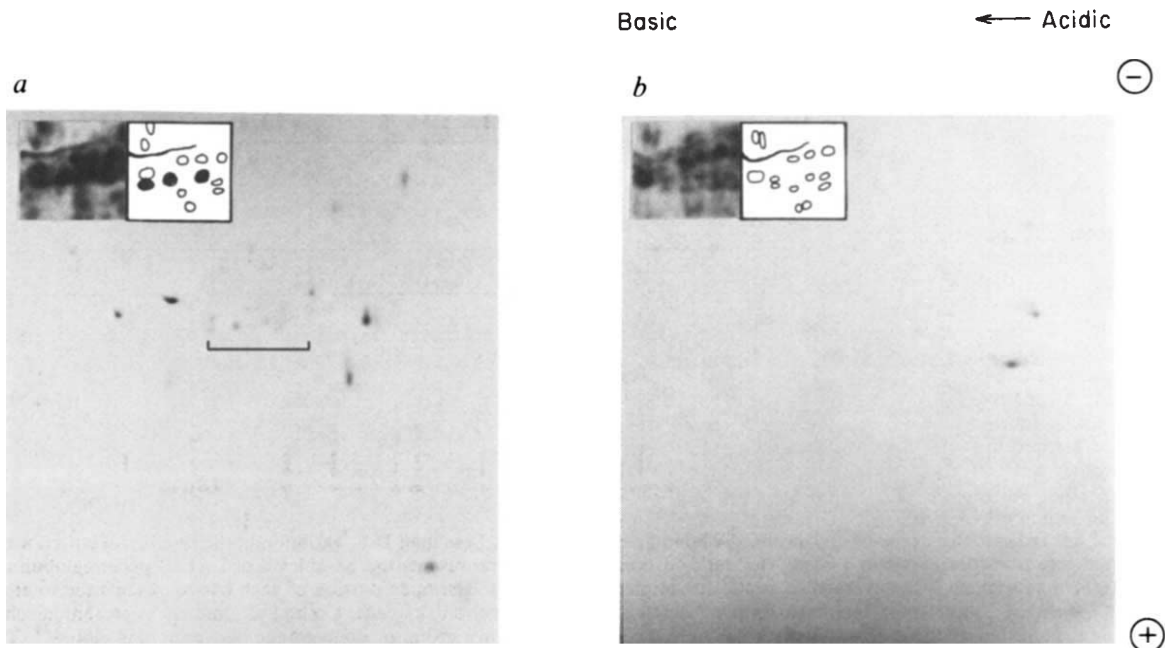


Fig. 7 Fluorographs of two-dimensional gels of the products of the c2.1 gene immunoprecipitated from K8-1 cells (a) and tk⁺ transformants (b) using C3H antibodies to K8-1 transformants. The antibodies prepared in C3H mice against c2.1 transformants (K8-1 cells) were used to immunoprecipitate radiolabelled antigens for two-dimensional gel electrophoresis as previously described¹⁸. The insets contain longer exposures and diagrammatic representations of the 45,000-molecular weight region of each gel. Black spots represent the specific immunoprecipitated molecules from 8-1 cells.

previously that the expression of the transferred genes is a stable phenomenon¹⁸.

The ability to measure an elevation in β_2 -microglobulin expression by the pooled transformants is related to the fact that in the conditions used for transformation, nearly 100% of the co-transformants express significant and comparable levels of the foreign class I molecules³⁶. It is important to stress that the twofold increase in the magnitude of the radioimmunoassay signal does not necessarily correspond with a twofold increase in the amount of β_2 -microglobulin expressed (see ref. 36). These results suggest that the L cells possess the capacity to express additional class I molecules. It has recently been shown that the expression of foreign class I molecules does not occur at the expense of the endogenous H-2^k products of the transformants³⁶. Furthermore, we have observed significant levels of expression of as many as three different products by the triple K^d, D^d and L^d cloned transformant line, which demonstrates even greater than a twofold increase in the β_2 -microglobulin radioimmunoassay signal.

At least 10 of the transformants which exhibited increased levels of β_2 -microglobulin expression failed to react with any of the known serological reagents to class I molecules (Fig. 5a,b), suggesting that these cells produced novel products associated with β_2 -microglobulin. Unfortunately, the heterologous antibodies to β_2 -microglobulin proved relatively ineffective in precipitating class I molecules for two-dimensional gel analysis. Thus, we decided to prepare antisera directly against several of these gene products in order to demonstrate formally the presence of novel class I genes. Mouse L cells transformed with the H-2L^d gene have been used to immunize C3H mice, the strain from which the L cell was derived, to generate alloantisera specific for the L^d molecule²⁰. Similarly, C3H mice were immunized with L-cell transformants (K8-1) obtained by gene transfer with cosmid clone 2.1, the first gene on cosmid cluster 1 (see Fig. 5a). By radioimmunoassay, the antibodies specific for K8-1 cells reacted weakly with BALB/c spleen cells, whereas significantly lower reactivity was observed against BALB/c liver or thymus cells, and C3H spleen, liver or thymus cells (Fig. 6). Accordingly, these antibodies constitute an alloantiserum that was then used to screen spleen cells from a panel of different congenic and recombinant congenic

strains of mice so that the location of gene c2.1 in the major histocompatibility complex could be assigned by preliminary mapping. These serological analyses suggest that gene c2.1 maps to the right of the complement region. More extensive mapping is required to pinpoint the location of this gene by serological analysis. Such analysis may be complicated if only certain strains of mice express the 2.1 gene products in a manner analogous to that of Qa or TL antigens. However, the data are consistent with the mapping of this gene by restriction enzyme polymorphisms, in that class I gene c2.1 is the first gene in the cosmid cluster containing gene 27.1, which had previously been mapped to the Tla region¹⁵ (Fig. 5a).

The antiserum to the K8-1 cells immunoprecipitated molecules from K8-1 cells but not from tk⁺ transformants or BALB/c spleen cells (Fig. 7). The products of the c2.1 gene appear to be ~45,000 molecular weight and to consist of a number of spots of the appropriate pI in a sequential array somewhat characteristic of class I molecules analysed on two-dimensional gels (see Fig. 2). β_2 -Microglobulin could not be readily detected in 8-1 immunoprecipitates. This may be due to a low affinity between β_2 -microglobulin and the 2.1 gene products, as has been described for certain H-2^d molecules³⁷. The origin of the additional products precipitated is unknown. The fact that these products cannot be isolated from spleen cells may be due to the fact that this antigen is expressed at low levels on lymphocytes. However, the ability to generate antibodies to the putative novel gene products expressed by the K8-1 transformants supports the existence of novel genes. Moreover, these genetic, serological and chemical data suggest that gene c2.1 encodes a class I molecule which may, because of its location within the major histocompatibility complex, be a differentiation antigen and not a ubiquitously expressed transplantation antigen.

Mapping of six serologically defined genes

The serologically defined class I genes have been localized within the major histocompatibility complex through the serological analysis of the highly polymorphic congenic mouse strains. Thus the identification of these class I genes by DNA-mediated gene transfer allows us to map precisely the corre-

sponding cosmid clusters into the major histocompatibility complex. It is interesting that only five class I genes shown in Fig. 5 map to the H-2 region (clusters 2, 11 and 13). Three class I genes encode defined transplantation antigens whereas the remaining two apparently do not encode detectable class I gene products. In contrast, 17 class I genes are contained in cosmid clusters which map to the Tla region. Four class I genes encode serologically defined differentiation antigens and eight encode novel gene products. One of the eight novel genes (c2.1) may encode a differentiation antigen based on the pattern of tissue distribution determined with the alloantibodies. Therefore, the Tla region contains far more class I genes than the H-2 region.

Cosmid clusters 3, 7, 8, 9, 10 and 12 do not express serologically defined gene products and, accordingly, cannot be mapped within the major histocompatibility complex (Fig. 5b). These six cosmid clusters contain 15 class I genes, six of which appear to express novel gene products. These clusters are now being mapped through the analysis of restriction enzyme site polymorphisms.

Conclusion

We have identified at least one H-2 K^d, D^d, L^d, possibly a Qa-2,3, and at least two TL genes from the BALB/c mouse.

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These genes have been mapped into 6 cosmid gene clusters containing 14 class I genes (Fig. 6), of which 9 lie in the Tla region and 5 in the H-2 region. As many as 10 putative novel class I gene products, detected on the basis of their association with β_2 -microglobulin, may be encoded by genes dispersed between the mapped and unmapped cosmid gene clusters. In one case, antibodies to a novel class I antigen apparently detect the corresponding antigen expressed at low levels on BALB/c spleen cells.

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LETTERS TO NATURE

Measurements on a shock wave generated by a solar flare

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Shock waves generated by intense solar flares may be driven by a large amount of ejected mass, about 5×10^{16} g, and the total energy involved may be of the order of 10^{32} erg. The shocks may have initial velocities of the order of $2,000 \text{ km s}^{-1}$ and, in their exodus through the corona, may be accompanied by fast-moving optical transients, the emission of highly characteristic radio signatures and the acceleration of particles to quasi-relativistic velocities¹. Here we review data on a high-velocity shock generated by a flare on 18 August 1979, 1400 UT, comment on some previously deduced velocities for the shock², and then present a model, based on current computer programs to account for the overall characteristics of the shock as it propagated through the corona and the interplanetary plasma.

The solar flare that occurred on 18 August 1979 at 1400 UT was one of the more energetic flares of the current solar cycle. Optically, the flare was not well observed because it occurred just behind the east limb at heliographic latitude N08°. Nevertheless, it gave rise to significant X-ray and radio emission, as well as to a high-velocity shock. The shock was tracked by its radio emission of spectral type II, first by radio spectral equipment (25-580 MHz) at the Harvard Radio Astronomy Station, Fort Davis, Texas, and then by low-frequency equipment (0.03-2 MHz) on the ISEE 3 satellite³. It impinged on the Earth's magnetosphere some 40 h later, giving rise to the sudden commencement of a magnetic storm. Data on the outward-travelling shock were also acquired at a point $13.1 R_0$ from the Sun by a technique described by Woo and Armstrong². Their velocity estimates were obtained by examination of the effects of the shock and its driver-piston on signals transmitted at 2.3 and 8.4 GHz from the Voyager 1 spacecraft and received on Earth. At the time of the flare, the line of sight from Voyager 1 to Earth passed within $13 R_0$ of the Sun and, when the shock passed this point, abrupt changes began in the radio signals received from the spacecraft, as may be seen in Fig. 1.

In applying the Rankine-Hugoniot relations to determine the shock velocity at $13 R_0$, Woo and Armstrong suggested using a pre-shock velocity of 200 km s^{-1} , with which we agree,

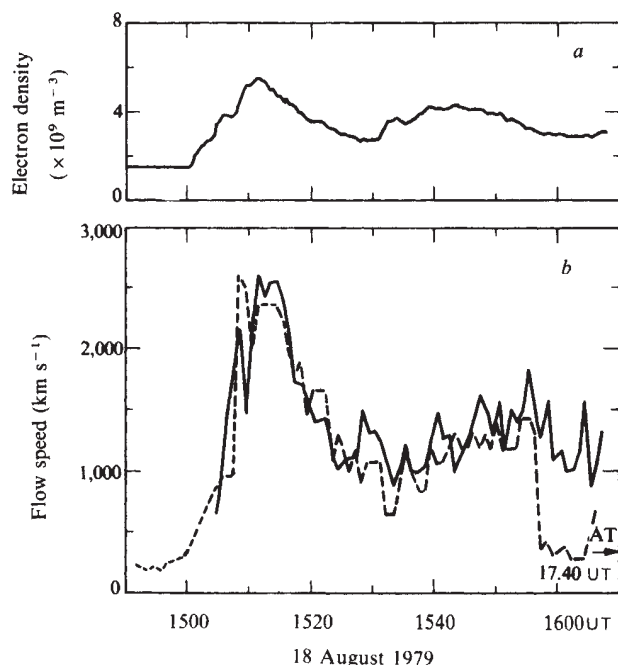


Fig. 1 Plasma data at $13.1 R_{\odot}$ ($R_{\odot}$ = radius of the Sun) derived from changes in radio transmissions from Voyager 1, at the time of passage of the flare-generated shock. *a*, Changes in electron density; *b*, changes in flow speed (after ref. 2). Dashed line, log amplitude scintillations; solid line, spectral broadening.

and a post-shock velocity of $2,600 \text{ km s}^{-1}$, which, based on the data of Fig. 1, we believe to be too high. We suggest that the flow velocity of $2,600 \text{ km s}^{-1}$ measured at 1510 UT by Woo and Armstrong refers to the plasma velocity substantially behind the shock, whereas the proper application of the Rankine-Hugoniot relations requires use of a velocity immediately behind the shock. We suggest the shock probably passed the $13 R_{\odot}$ point at 1500 UT, that its passage probably took $< 10 \text{ s}$, and that everything seen thereafter in Fig. 1 refers to the region of intense turbulence behind the shock. The 10-s period also represents an upper limit and is derived on the assumption that the shock thickness is, at most, of the order of 1–10 ion gyroradii^{4,5}. In view of our ignorance of magnetic field strength and direction in the shock, we use a roughly equivalent estimate of 10–100 ion inertial lengths^{4,5}. Using the pre-shock electron density of $1.5 \times 10^9 \text{ electrons m}^{-3}$ given by Woo and Armstrong, and assuming that the electron density equals the ion density, we find the ion inertial length (c/ω_{pi} , where c is the velocity of light and ω_{pi} , the ion plasma frequency) to be 6 km and hence deduce the shock thickness to be of the order of 60–600 km. For a shock travelling at about $2,000 \text{ km s}^{-1}$ the front would thus pass the line of sight in $\sim 1 \text{ s}$ —literally in a flash. We therefore choose the period 1502–1504 UT in Fig. 1, to determine the post-shock velocity, which would thus be of the order of $1,000$ – $1,500 \text{ km s}^{-1}$. Assuming conservation of mass through the shock and the pre-shock electron density and velocity given by Woo and Armstrong, we then determine the shock velocity as follows. Assume that the pre-shock velocity $v_1 = 200 \text{ km s}^{-1}$, the post-shock velocity $v_2 = 1,250 \text{ km s}^{-1}$, the pre-shock electron density $n_1 = 1.5 \times 10^9 \text{ electrons m}^{-3}$, and the post-shock electron density $n_2 = 3.0 \times 10^9 \text{ electrons m}^{-3}$. The shock velocity then equals $(v_2 n_2 - v_1 n_1)/(n_2 - n_1) = 2,300 \text{ km s}^{-1}$ approximately, rather than the value of $3,500 \text{ km s}^{-1}$ derived by Woo and Armstrong.

In a second estimate of the shock velocity, based on its transit time from the flare region to $13 R_{\odot}$, Woo and Armstrong noted that “shock arrival occurs at approximately 1501 UT and based on the transit time from the Sun to $13.1 R_{\odot}$ we obtain a shock speed of $3,509 \text{ km s}^{-1}$ ”, but they made no specific reference to the starting time of the shock in the vicinity of the solar flare.

There are, however, four separate lines of evidence that indicate that the explosive phase of the flare, which we take to be the starting time of the shock, occurred at approximately 1402 UT. A transit time of 60 min for a shock to reach $13 R_{\odot}$ then translates into an average velocity of $2,350 \text{ km s}^{-1}$. The evidence relating to the explosive phase of the flare, shown in Fig. 2, is as follows: (1) an abrupt increase in radio centimetric emission began at approximately 1402 UT and this was followed by a burst of 45 min duration and of considerable energy. (2) Hard X-ray emission showed a sudden increase over the period 1359–1401 UT. (3) A metric radio type II burst was recorded over the period 1412–1423 UT and statistical surveys repeatedly show that type II bursts in the metre band are first seen about 6–10 min after the explosive phase of a flare⁶ (this delay corresponds to the time taken by a shock to travel from the region of energy release in the low corona to the heights at which metric type II radio bursts are generated), which again suggests a start time for the shock of about 1402 UT. (4) Type III radio bursts, probably associated with the explosive phase of the flare, were recorded at 1402–1404 UT. (Figure 2 also shows a small radio burst in the centimetre band and an accompanying X-ray burst at 1345 UT. We believe, as do other investigators (S. Kane personal communication), that this burst was a precursor to the main event. If it did, however, signify the start time of the shock, the transit time of the shock to $13 R_{\odot}$ would be 15 min longer and the deduced average shock velocity would be even less than $2,350 \text{ km s}^{-1}$.)

A shock velocity of $2,300 \text{ km s}^{-1}$, rather than the velocity of $3,500 \text{ km s}^{-1}$ derived by Woo and Armstrong, agrees somewhat better, we feel, with existing models for propagation of flare-generated shocks from the Sun to the Earth. For this particular flare, the propagation time of the shock between the Sun and the Earth was 40 h. (The shock reached the ISEE 3 satellite, located at the lagrangian point between the Sun and the Earth,

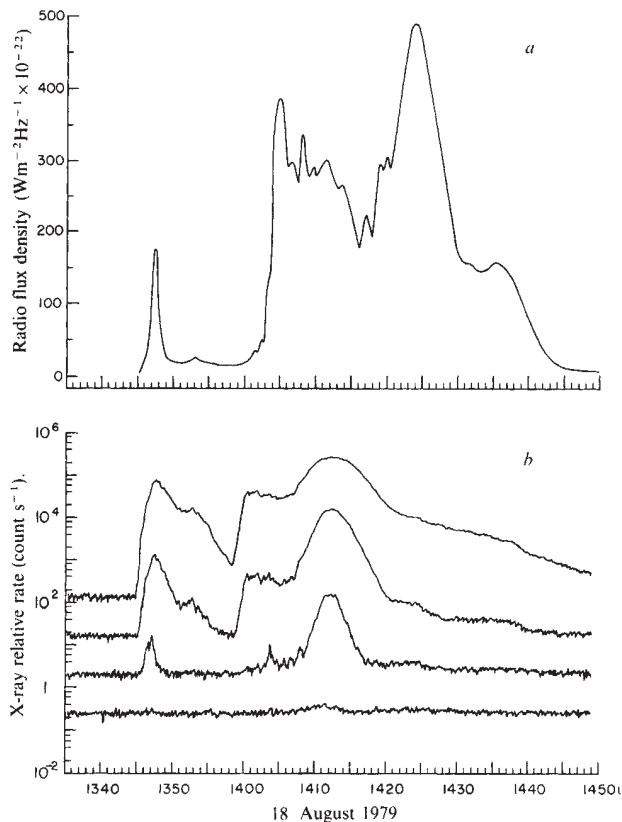


Fig. 2 Radio and X-ray emission from E-limb solar flare of 18 August 1979, 1400 UT. *a*, Radio burst at 2,800 MHz. *b* X-ray bursts recorded by ISEE 3 satellite; reading from top to bottom, the X-ray curves cover the energy ranges 26–43, 43–78, 78–154 and 154–398 keV, respectively.

on 20 August 1979 at 0550 UT² and the Earth at 0625 UT.) Bearing in mind that the flare occurred at heliographic longitude $\sim$ E 90, we suggest that, to reach the Earth, the shock must have had a wide angular extent and very considerable energy. We suggest that it had an initial velocity of the order of $2,300 \text{ km s}^{-1}$ and that, while ascending through the corona it was accelerating electrons to quasi-relativistic energies and generating X rays (identifiable in Fig. 2 by the marked increase in X rays over the period 1407–1420 UT, at the time of the observed type II radio burst). The shock was then driven at a constant velocity of $\sim 2,300 \text{ km s}^{-1}$ to a distance of about $75 R_{\odot}$ (S. Kane, personal communication), after which it propagated through the solar wind to the Earth as a blast wave, reaching the Earth $\sim 40 \text{ h}$ after the flare with a velocity of 750 km s^{-1} . The average deceleration between 75 and $215 R_{\odot}$ was thus 0.012 km s^{-2} , which is consistent with previously estimated values for the deceleration of shocks between the Sun and the Earth^{7,8}. We also note that the wide helio-longitudinal extent of the shock probably represents the rule, rather than the exception, for flare-generated shocks.

The X-ray data in Fig. 2 are reproduced by kind permission of Dr Sharad Kane, Space Sciences Laboratory, University of California, Berkeley. The centimetric radio data in Fig. 2 were provided by courtesy of Dr M. B. Bell, Astrophysics Branch, National Research Council, Ottawa. The work of Alan Maxwell described in this paper was carried out under NASA grant NSG-7648 and NSF grant ATM 80-07024.

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Coronagraphic observations of two new sungrazing comets

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We recently reported the discovery¹ of a comet (Howard-Koomen-Michels: 1979 XI) that apparently collided with the Sun on 30 August 1979. We now report observations of two additional sungrazers that encountered the Sun on 27 January 1981 and 20 July 1981, respectively. Like comet 1979 XI, these two new comets seem to have been members of the Kreutz group of sungrazers²⁻⁴, and like 1979 XI the new comets did not reappear after their encounters with the Sun. The discovery of three previously unreported comets during the initial 2.3 yr of our satellite coronal observations leads us to suspect that sungrazers are much more common than one might suppose from the list of only nine known sungrazers observed during the years 1668–1970^{5,6}.

Our observations were obtained with the Naval Research Laboratory's Earth-orbiting SOLWIND coronagraph. This instrument has been operating routinely on the US Air Force Space Test Program satellite, P78-1, since March 1979. Its broadband (4,100–6,350 Å) images show a Sun-centred annular field of view from 2.5 to $10.0 R_{\odot}$ with a spatial resolution (pixel separation) of 1.25 arc min and with sensitivity adequate to record features having more than 10^{-10} times the brightness of the solar photosphere. In its normal operating mode, SOLWIND has obtained full-frame coronal images at 10-min intervals during the 1 h sunlit portion of each 97-min

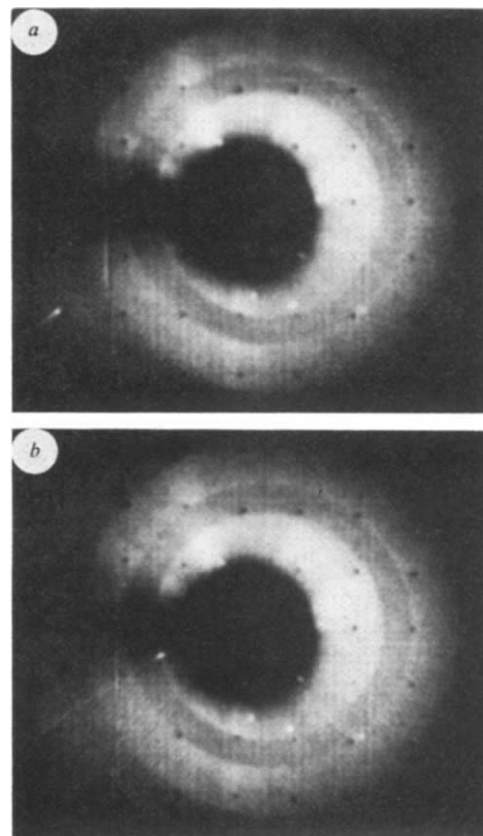


Fig. 1 The comet as seen against a background of the quiescent solar corona. *a*, The first frame in which the comet was detected, was recorded by the SOLWIND satellite coronagraph at 2039 UT on 26 January 1981. It shows the coma at a $7.9 R_{\odot}$ projected distance from the Sun's centre. Subsequently, the coma dimmed noticeably (see Fig. 2 and discussion in the text), but it flared brightly again in *b* at 0020 UT 27 January 1981, just before passing behind the coronagraph's occulting disk, at $2.5 R_{\odot}$. The dark, nearly concentric rings surrounding the occulter shadow are polarization analysers in the instrument's focal plane. Solar north is at the top in each frame, and east to the left.

satellite orbit. This instrument and its initial observations have been discussed in detail elsewhere^{1,7,8}.

In Fig. 1, the coronal images at 2039 UT 26 January 1981 and 0020 UT 27 January 1981 show the second of the three comets that we have observed with the SOLWIND coronagraph. The bright coma and fainter tail are visible as the comet approached the Sun from the south-east (lower left). In each image, the central dark area is the shadow of the instrument's occulting disk that artificially eclipses the Sun, and the dark region extending to the left is the shadow of the occulter support. The two dark, nearly concentric rings surrounding the occulter shadow are polarization analysers in the focal plane. They pass light that is polarized only in the radial direction, while the remaining regions pass light that is polarized only tangentially, thus favouring the Thomson-scattered light from free electrons in the Sun's *K*-corona. Note that the comet's light does not appear to be polarized, but that the bright coronal streamer in the north-east (upper left) is strongly blocked by the analyser rings.

We obtained 15 such images of this comet as it approached the Sun at a projected speed of 250 km s^{-1} before disappearing behind the occulting disk. Figure 2 shows a partial sequence of these images during 2039 UT 26 January–0030 UT 27 January 1981, and also includes an image at 0654 UT 27 January by which time the comet's nucleus should have reappeared from behind the occulting disk. In each image, the white disk was introduced to indicate the approximate size and location of the occulted photosphere. Also, unlike the images in Fig. 1,

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these images in Fig. 2 are difference images that show only intensity changes since 1300 UT on 26 January, before the comet's entrance into the field of view.

The temporal variation of the coma's apparent brightness is clearly visible in Fig. 2. Beginning around 2039 UT, the

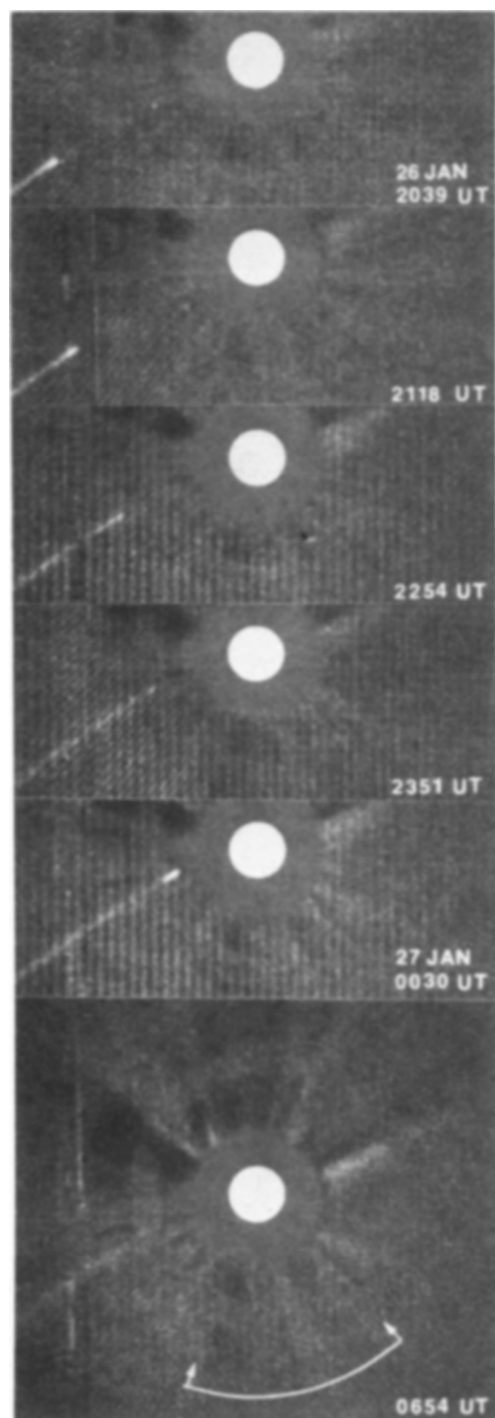


Fig. 2 Variations in the brightness of the coma are easily discernible in the first five frames, showing approach of the comet to perihelion. The sixth frame, after perihelion, shows a wider portion of the field at 0654 UT on 27 January. The arrows indicate a region in the corona through which the comet would have passed, had it survived perihelion, but no vestige of the coma was observed after the frame at 0030 UT 27 January. The photos in this sequence are difference images, showing only changes in the coronal scene after about 1300 UT on 26 January. A white disk has been added to each frame to indicate the location and size of the Sun, which is hidden by the coronagraph's occulting disk. The radial light and dark spoke-like features represent evolutionary increases and decreases, respectively, in the intensity of coronal streamers.

apparent brightness decreased steadily until 2351 UT after which it increased substantially as the comet came within a few radii of the Sun. Although we have not yet performed quantitative photometry, we think that the initial brightness decrease is a real effect and not simply the result of the radial decrease of the instrument's transmission function as one approaches the Sun. A comparison with stars and planets of known brightness suggests that the coma's brightness changed from a magnitude of roughly 0.0 at $8 R_{\odot}$ to +1.0 at $5.5 R_{\odot}$, and finally to -2.5 or brighter at $3 R_{\odot}$. Thus, unlike our first comet (1979 XI) whose magnitude reached approximately -3.5, this second comet was relatively faint during its initial approach to the Sun. This may be why it has not been reported previously despite the optimal position of its orbit relative to the Earth in the months before 26 January 1981. Of course, the fact that the comet did not reappear after perihelion accounts for why it was not observed in the months following 27 January 1981. Indeed, the sudden flare up of brightness around 0030 UT 27 January may indicate the beginning of the comet's final demise as it neared the Sun.

The 15 pre-occultation images were obtained during a scant 231-min interval with a spatial resolution (pixel separation) of 1.25 arc min and with modest absolute positional errors associated with image distortion and uncertainties in spacecraft roll angle. Consequently, it is difficult to determine the orbital elements uniquely, or even to distinguish between a retrograde orbit (motion counter to that of the planets around the Sun) or a so-called direct (prograde) orbit. Nevertheless, using the same technique that we applied to the observations of our first comet (1979 XI)¹, we have been able to find a small range of possible orbits (as a function of perihelion distance as a parameter) that fit the 15 pre-occultation observations within our

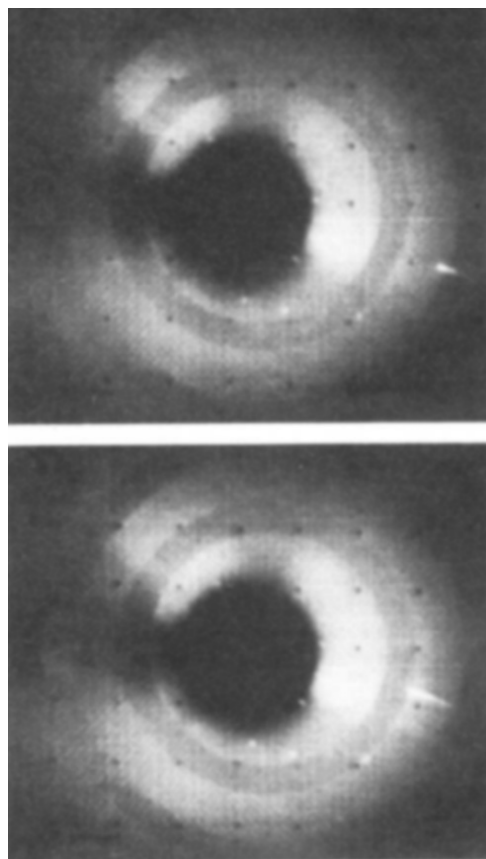


Fig. 3 Coronagraph images of a sungrazing comet as it approached the Sun from the south-west at 0209 UT and 0354 UT 20 July 1981. The format is the same as that in Fig. 1 with solar north approximately up and west approximately to the right. Unlike coronal streamers, whose polarized Thomson scattered emission is cut off by the dark polarizing rings, the comet is largely unpolarized and remains bright as it passes across the rings.

measurement accuracy. A retrograde orbit having a perihelion distance of $\sim 1.5 R_{\odot}$, a perihelion time of 0230 UT 27 January, and orientation angles representative of the Kreutz group is consistent with our observations. [Marsden⁹ has used our measurements to deduce a perihelion distance of $1.05 R_{\odot}$, a perihelion time of 0149 UT 27 January 1981, and the orbital angular elements $\Omega = 350^{\circ}$, $\omega = 72^{\circ}$, and $i = 143^{\circ}$ assuming that the comet's perihelion direction agrees with that of the Kreutz group.]

In Fig. 2, the area marked by arrows on the image at 0654 UT shows the region through which the comet would have moved outward from the Sun during the morning of 27 January 1981 if its perihelion distance lay in the range $1-2 R_{\odot}$. Regardless of which of these representative orbits is assumed, the outbound coma should be visible in this image, but it is not. Nor was it visible on any other image obtained during 0001-1100 UT on 27 January 1981. Evidently, the comet either hit the Sun or was destroyed (or at least purged of its volatile material) in a close encounter. However, this does not explain why the 'outbound' tail was not visible after perihelion as it was with comet 1979 XI which we suppose hit the Sun¹. At 0654 UT one can see only the fading trace of the tail along the inbound track together with some obvious coronal streamer changes, especially prominent in the Sun's northern hemisphere. It is possible that an 'outbound' tail, if it existed, would be undetectable for this relatively faint comet.

Figures 3 and 4 show similar observations of the third sungrazing comet that we have seen with the SOLWIND coronagraph. Figure 3 shows that the head of this comet is not cut off by the polarizing ring so that, like the first two comets, it is largely unpolarized. By comparison with a previously recorded image of the planet Mercury on 28 December 1980, we estimate that this third comet had a magnitude of -0.8 at $8 R_{\odot}$.

More than 15 images were obtained before occultation on 20 July 1981, and numerous additional images were obtained during the subsequent day. The comet approached the Sun with a projected speed of $\sim 160 \text{ km s}^{-1}$ during 0029-0354 UT. As for the first two comets, our analysis of these images gave an orbit, which, if assumed to be retrograde, is consistent with those of the Kreutz group, this time with a perihelion of $\sim 1.2 R_{\odot}$. Its perihelion time would have been 0805 UT 20 July, nearly the time of the fifth image in Fig. 4 in which the arrow marks the uneclipsed end of the comet's gradually lengthening tail. [Marsden¹⁰ deduced a similar orbit with a perihelion distance of $0.9 R_{\odot}$, a perihelion time of 0742 UT 20 July, 1981, and orbital angular elements $\Omega = 350^{\circ}$, $\omega = 74^{\circ}$, and $i = 143^{\circ}$ again assuming that the perihelion direction agrees with that of the Kreutz group.] We suppose that the relatively short, tear-drop shape of this comet's tail is due in part to a perspective effect associated with the more oblique (head-on) orientation of the orbital axis relative to the line of sight than was obtained for the first two comets.

Finally, like our first two comets, this third sungrazer never reappeared after its occultation, and like the second comet (Fig. 1) no post-perihelion dust cloud was visible. In Fig. 4, the difference image at 2225 UT shows only coronal enhancements and depletions that have occurred in the 24 h since 2200 UT 19 July. The greatest enhancement lies in the south-east where the comet's head should have reappeared if its perihelion were in the range $1-2 R_{\odot}$. However, this enhancement represents the accumulated evolution of a slowly brightening coronal structure whose initial growth is faintly visible in the earlier images in Fig. 4 well before this comet reached the Sun.

Perhaps the importance of these new observations is to suggest that sungrazing cometary encounters may be relatively frequent. Before the beginning of SOLWIND operation in March 1979, nine Kreutz sungrazers had been reported during the years 1668-1970. Since March 1979, we have seen three additional sungrazers despite the fact that we have processed coronal images for only 500 days to date (July 1982). Significantly, none of these three sungrazers was seen from the ground¹¹. Evidently the SOLWIND coronagraph is observing

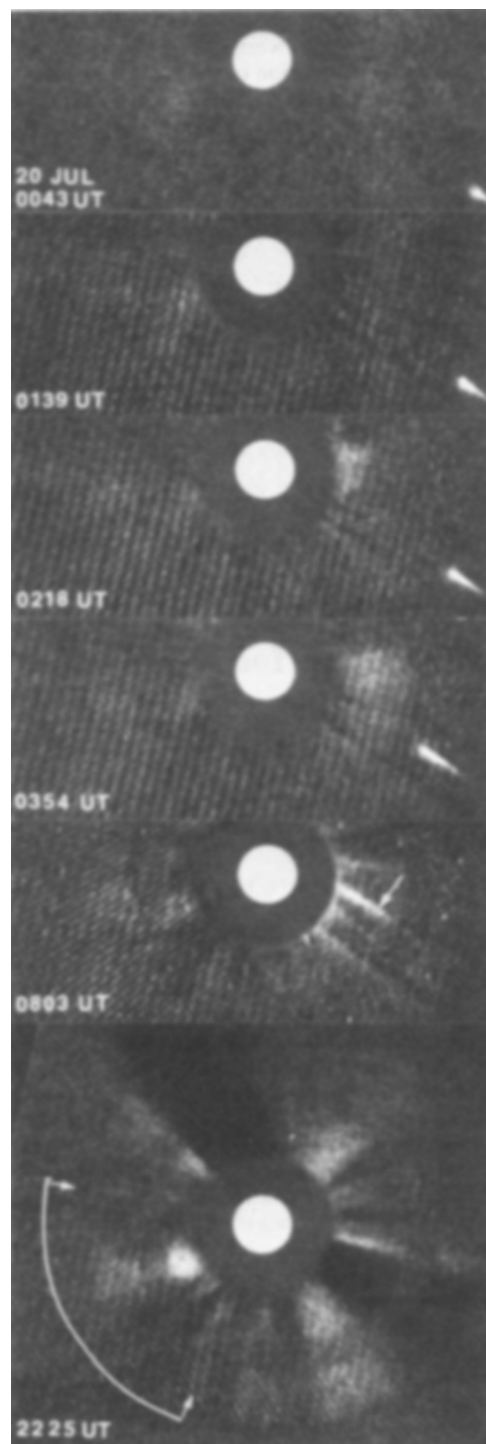


Fig. 4 A sequence of difference images showing intensity changes in the coronagraph field of view since 2200 UT 19 July 1981. Solar north is up and west is to the right. The inbound comet is clearly visible during 0043-0354 UT 20 July as it approached the Sun from the south-west. Its tail is still visible at 0803 UT (arrow) by which time the head has passed behind the occulting disk. By 2225 UT, there is no trace of either head or tail among the numerous increases and decreases of coronal intensity. The greatest enhancement, visible in the south-east where the comet's head is expected to reappear (arrows), represents the accumulated brightness increase of a coronal structure whose initial brightening is already faintly visible in the pre-occultation frames of this figure. The white disk at the centre of each image indicates the apparent size and location of the occulted photosphere.

the faintest and perhaps most perishable fragments of the supposed primordial comet whose close encounter with the Sun may have led to the formation of the Kreutz group.

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A dispersion law for solar oscillations

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The pressure or *p*-modes are acoustic vibrations trapped in a resonant cavity below the solar surface. The waves are most easily observed as primarily vertical velocities at the solar surface by Doppler shift techniques. The vertical velocities vary harmonically in time and in space across the solar surface. These harmonic variations lead to a natural description of the oscillation modes in terms of their positions in a diagram of temporal frequency (ω) versus horizontal spatial frequency (k_h). Observationally, the positions in the k_h - ω diagram are determined from a two-dimensional power spectrum of a series of Doppler shifts of a solar spectrum line measured equidistant in space and time. An example of the observed positions is shown in Fig. 1 (see ref. 1). Theoretically, the mode positions are determined by an analysis of the solar cavity, including an uncertain model of the solar interior. Most theoretical work has consisted of numerical solutions of the equations of motion leading to predicted positions in the k_h - ω diagram (see, for instance, ref. 2). This approach has been reasonably successful, yielding inferences about the depth of the convection zone³. Here we compare a simple model of the oscillations discussed by Leibacher and Stein⁴ with observations.

In the Leibacher and Stein⁴ model the resonant cavity is a spherical shell. To first order the outer boundary of the shell is considered to be the same for all modes and is defined by the sharp decline in the density scale height just below the visible surface. The inner boundary of the shell is caused by the increase of sound speed with depth. Waves travelling downwards at an oblique angle to the vertical (with non-zero horizontal wavenumber k_h) are refracted away from the vertical by the increasing sound speed. At some depth the propagation becomes horizontal and the wave is reflected, this marking the lower boundary of the cavity. At this depth the sound speed is equal to the horizontal phase velocity ω/k_h . Leibacher and Stein³ argue that the boundary conditions should be similar to an organ pipe closed at one end and open at the other. The WKB approximation is then used to derive the condition:

$(n + 1/2)\pi/\omega = \int dz/c$. Where z is the vertical coordinate, c is the speed of sound and n is the number of nodes in the vertical standing wave patterns. The integral is over the depth of the cavity. $\int dz/c$ is the travel time of sound across the cavity. The constant $1/2$ depends on the choice of boundary conditions⁵. The different curves in Fig. 1 correspond to different values of n while distance along a given curve corresponds to cavities of different depths.

From this simple model we draw two conclusions. (1) If the top of the cavity is the same for all modes and the bottom of the cavity is defined by the position where $c = \omega/k_h$, then modes in different parts of the k_h - ω diagram with the same value of ω/k_h are trapped in an identical cavity. (2) If the cavity is identical, the travel time of sound across the cavity will be the same for different modes. Therefore, in a plot of ω/k_h (an observed quantity) versus $(n + 1/2)\pi/\omega$ (another observed quantity), the ridge structure of Fig. 1 should collapse onto a single curve. This leads to a modified dispersion law of the form $(n + 1/2)\pi/\omega = f(\omega/k_h)$.

Using this procedure we found that the ridge structure did not collapse onto a single curve. To admit the possibility of other boundary conditions similar plots were constructed, but with the ordinate given by $(n + \alpha)\pi/\omega$, for different values of α . It was found that near $\alpha = 3/2$ the points collapse onto a single curve, within the observational accuracy (Fig. 2). To determine a best value for α the residual variances to fifth-order polynomial fits to the resulting points was computed as a function of α . The minimum residual variance (or the tightest curve) was found for the value $\alpha = 1.50$. The standard deviation of the points about the polynomial fit is 1% of the mean value of $(n + 3/2)\pi/\omega$. The fundamental mode ($n = 0$) has been excluded from this analysis and from Fig. 2 as its frequencies do not fall along the curve of Fig. 2.

An important aspect of the above result is the reduction of the redundancy in the k_h - ω diagram. Essentially all of the information about the solar interior contained in the multiple curves of Fig. 1 is concentrated in the single curve of Fig. 2. Modes trapped in an identical cavity are giving redundant information, and the result of Fig. 2 strongly suggests that modes with the same value of ω/k_h are trapped in an identical cavity. This is surprising, as the assumption of an identical boundary at the top for all modes is really a gross simplification of the problem. The upper boundary should really depend on

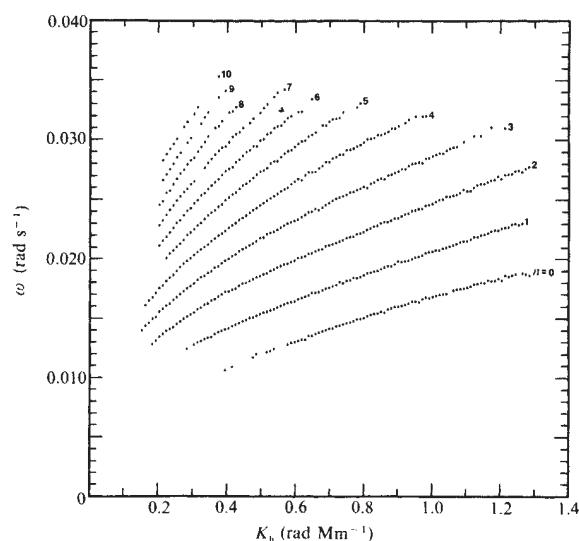


Fig. 1 Mode frequencies derived by a centroid analysis from a two-dimensional (ω, k_h) power spectrum of velocity observations made at Kitt Peak in May 1980 (ref. 1). The curves are labelled with the radial harmonic number n . Solar rotation has been cancelled by averaging the frequencies ω for eastward and westward propagating waves.

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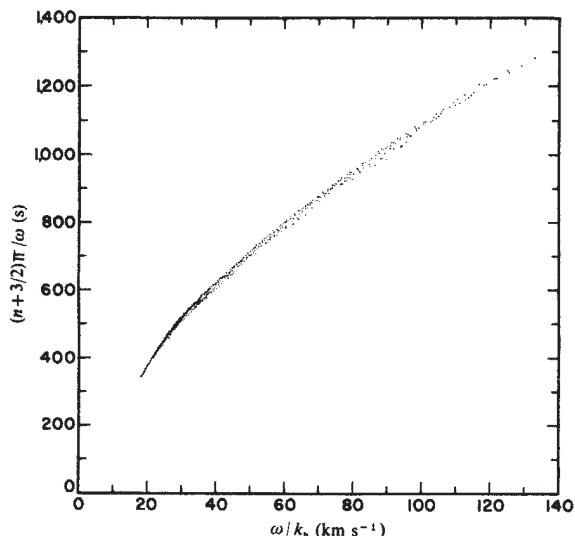


Fig. 2 Plot of $(n + 3/2)\pi/\omega$ against ω/k_h for the mode positions of Fig. 1. The $n = 0$ modes are excluded.

ω (ref. 6). The above result suggests that the difference in boundary conditions at the top for different modes has a minimal effect on the resulting frequencies.

To within the observational accuracy the best value of α is $3/2$. This may be fortuitous, but it does admit the possibility that the Leibacher and Stein analysis is correct and that we have misidentified the radial node number n . This seems unlikely because of the general agreement between the theory and observed frequencies. However, n is not observed directly but is inferred from this general agreement. So n misidentification is possible though unlikely.

Another possible source of the value $\alpha = 1.50$ is the atmospheric structure. Christensen-Dalsgaard⁷ has shown that for a polytrope of index m the frequencies obey a relation $\omega/k_h[(n + m/2)/\omega]f(n, m)$, where $f(n, m)$ has a weak dependence on n and a dependence on other atmospheric structure parameters. If we could assign a polytropic index $m = 3$ for the solar convection zone, we could approach the result of Fig. 2. However, the polytropic index is a strong function of position in the upper convection zone according to current models. Also, the function $f(n, m)$ for $m = 3$ introduces differences between modes of different n that are larger than the observed scatter in Fig. 2. Although the polytropic model may not be directly applicable, it does point out a way of getting a dispersion law that is dependent on the atmospheric structure.

We have considered whether the data might be better fitted by an α which is a function of another parameter, possibly ω/k_h or ω . However, the data are fitted to the observational accuracy by a constant α and so we have not felt justified in introducing further dependencies.

If the ordinate of Fig. 2 is the travel time of sound across the cavity and the abscissa the speed of sound at the bottom of the cavity, the data of Fig. 2 could be directly inverted to obtain the speed of sound plotted against depth. We are investigating this possibility.

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Critical density fluctuations within a single polymer chain

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The coil-globule phase transition is a reversible, conformational change of a macromolecule where the high temperature (or good solvent) phase is an extended coil and the low temperature (or poor solvent) phase is a tightly packed globule¹. We now present direct observations, made by photon-correlation spectroscopy, of the sharp increase in amplitude and relaxation time of density fluctuations within a single polymer molecule as it passes through the coil-globule phase transition. This growth of fluctuations is characteristic of critical phenomena associated with phase transitions, a subject that has generated much interest over the past decade, and has been studied in a wide variety of physical systems. These are the first measurements of critical behaviour that we know of made on a three-dimensional system that can be characterized as finite in the terms of statistical mechanics.

The phase transition is a manifestation of competition between two forces acting on the polymer chain. Affinity among polymer segments tends to shrink the polymer, while an entropic pressure originating from the thermal motion of the individual segments tends to expand it. At higher temperatures

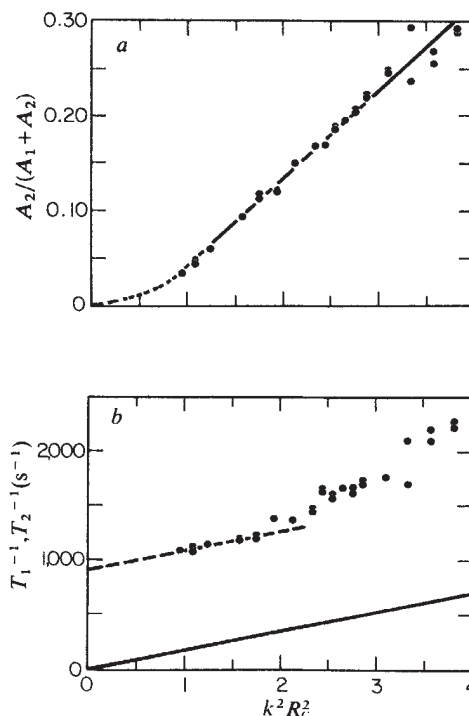


Fig. 1 Characteristics of intramolecular fluctuations at 33.9°C plotted as a function of the parameter $x = k^2 R_G^2$. a, The normalized amplitude. b, The frequency of internal motion. The solid line is the frequency associated with the translational diffusion. Points representing the frequency of the lowest order mode of internal motion fall parallel to the solid line at small values of x .

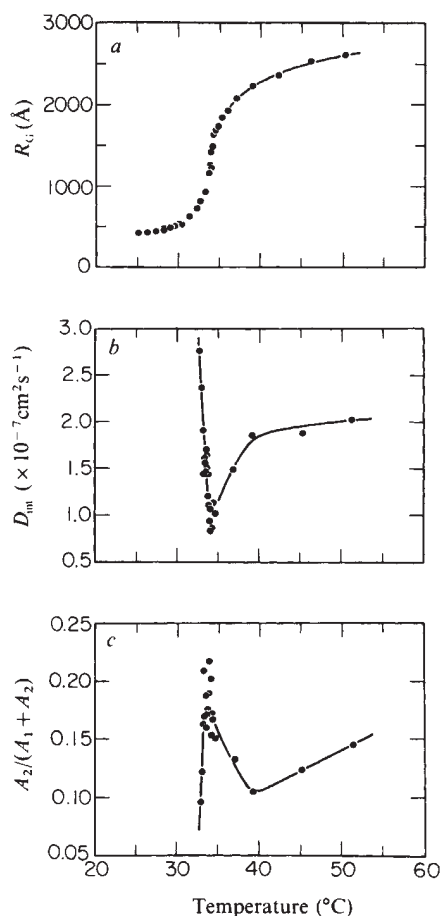


Fig. 2 The behaviour of the intramolecular fluctuations as a function of temperature (lines to guide the eye). *a*, The collapse seen in the radius of gyration. *b*, The internal diffusivity of the segments, $D_{\text{int}} = R_G^2(T_2^{-1} - T_1^{-1})$. *c*, The amplitude of the fluctuations at $x = 2.5$.

(or in a good solvent) the entropic pressure dominates the segment affinity, and the polymer is expanded. At lower temperatures (or in a poor solvent) the segment affinity is dominant, and the polymer collapses. For flexible polymers of large molecular weight the collapse is quite drastic²⁻⁴. The polymer conformation is not frozen in during the process of collapse, however. Individual segments along the polymer backbone undergo thermal motion resulting in intramolecular concentration fluctuations. The brownian diffusion of the macromolecules as a whole through the solvent also creates concentration fluctuations, but on a longer time and length scale. The autocorrelation function of light scattered from the polymer solution contains information from both sources. The amplitude and relaxation time of intramolecular fluctuations can be determined by careful analysis of the correlation function at various scattering angles.

The polymer-solvent system used in the experiment is polystyrene (Polysciences $\bar{M}_w = 2.6 \times 10^7$, $\bar{M}_w/\bar{M}_n = 1.3$) in cyclohexane ($T_\theta = 35.4^\circ\text{C}$). The light scattering apparatus is of typical construction. The sample solution's temperature stability could be maintained to within a couple of mK over a period of several days. This is especially important in the region of the transition, where polymer size changes drastically with temperature. The sample was sufficiently dilute ($\sim 10^{-6}\text{ g ml}^{-1}$) so that the collapsing polymers would remain dispersed without aggregation.

Different length scales can be probed by varying the angle of collection of photons scattered by the polymer solution. The length scale is determined by the inverse of the scattering vector, $k = (4\pi/\lambda) \sin(\theta/2)$, where λ is the incident wavelength in the

solution, and θ is the scattering angle. A useful parameter for characterizing the length scale is $x = k^2 R_G^2$, where R_G is the polymer radius of gyration. At forward scattering angles where $x < 1$, only length scales larger than the molecular size are probed; the correlation function is a single exponential,

$$g^{(2)}(t) \propto \exp(-2Dk^2t) + \text{constant}$$

where D is the translational diffusion coefficient of the polymer. Shorter distances are probed in the range $1 < x < 4$. The lowest internal mode representing fluctuations in the overall polymer size contributes to the correlation function, which is now described by two components,

$$g^{(2)}(t) \propto \exp(-2Dk^2t)\{P_0(x) + P_1(x) \exp(-t/\tau)\}^2 + \text{constant}$$

where τ is the relaxation time of the lowest mode^{5,6}.

An established procedure was used to characterize the internal motion^{7,8}. At each temperature, correlation functions were measured at forward scattering angles ($x < 1$) to determine D . The correlation function was then measured at the higher scattering angles and fit to

$$c(t) = \{A_1 \exp(t/T_1) + A_2 \exp(t/T_2)\}^2 + B$$

where T_1 is fixed by D . The parameters A_1 , A_2 , T_1 , and B were varied.

Figure 1a shows the behaviour of the normalized amplitude of the internal motion, $A_2/(A_1 + A_2)$, as a function of x at 33.9°C . Figure 1b shows the inverse relaxation time, T_2^{-1} , also as a function of x at the same temperature. The translational relaxation time T_1^{-1} is plotted as a straight line through the origin in Fig. 1b. The value of τ is obtained from the difference in T_1^{-1} and T_2^{-1} in the region where they are parallel. For higher values of x , the effect of the higher-order modes corresponding to shorter length-scale density fluctuations is apparent—the two-exponential form is no longer appropriate to describe the measured correlation function. Figure 2a reproduces our previously published results showing the collapse in the radius of gyration as temperature is lowered⁴. Figure 2b is a plot of the diffusivity of the internal motion defined as $D_{\text{int}} = R_G^2/\tau$, derived from the relaxation time of the intramolecular fluctuations versus temperature. This combination of R_G and τ is appropriate since τ scales in proportion to R_G^2 , and because the proportionality coefficient D_{int} actually represents the intramolecular segment diffusivity^{5,9}. The slowing intramolecular motion is quite apparent in this plot. Figure 2c is the normalized amplitude $A_2/(A_1 + A_2)$ versus temperature at $x = 2.5$, indicating an approach to critical divergence.

More precise measurements of this phenomenon are underway. Future experiments that use carefully prepared monodisperse polymers in a range of molecular weights to measure the effect of the size of the statistical system on the critical behaviour would prove interesting. The physics of the polymer conformational fluctuations in the vicinity of the collapse phase transition may also prove useful in describing the function of certain biological macromolecules.

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Meandering of the subtropical front south-east of the Azores

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A subtropical front was observed in the area south and south-east of the Azores during cruises of *FS Meteor* and *FS Poseidon* in early 1982. The front has a basically west-east extension, with considerable meandering observed. Meso-scale eddies are found on both sides. The overall flow pattern corresponds to earlier results on geopotential differences in the upper north-east Atlantic, but the baroclinic transport of the order of $10^7 \text{ m}^3 \text{ s}^{-1}$ is found to be concentrated in a 60-km wide jet. We suggest here that the current band is part of the gyre circulation, resulting from a branching of the North Atlantic Current.

Such subtropical fronts can be expected where strong Ekman transport convergence occurs in a region of large horizontal thermohaline or haline gradients. They are temperature and salinity fronts in winter and spring and salinity fronts only in summer and autumn in the North Pacific¹, with a density front existing simultaneously at both times of the year. Jet-like baroclinic surface currents have been found at a 1,000-km long front in the central subtropical North Atlantic, with current speeds as high as 50 cm s^{-1} (ref. 2). The subtropical front is more pronounced in the Southern Hemisphere, and a complicated mesoscale structure connected to the front has been found in the southern Indian Ocean³.

The subtropical front also marks the transition between the eastward and westward flow in the oceanwide anticyclonic gyre circulation. Early studies of the North Atlantic circulation in the area north of the Azores^{4,6} led to the conclusion that the North Atlantic Current splits into two branches at approximately 45° N , 25° W , with a southern branch heading towards the Azores and continuing south-east towards Madeira. The upper ocean flow field can most easily be seen in the potential density field in Fig. 77 of ref. 6, indicating a splitting-up into two more branches west of the Azores. An eastern branch in this historical map flows southward to the Azores, then veers left towards the south-east. A second branch reaches the Azores west of the Mid-Atlantic Ridge, oriented west at first at 37° N and turns back to the east at 35° N . Note that Hansen's⁵ numerical model of Atlantic Ocean surface currents including a variable wind field produced a dynamic topography that is consistent with a flow from the Azores to the Madeira region. A similar basic pattern can be found in more recent maps of upper ocean geopotential differences⁷⁻⁹.

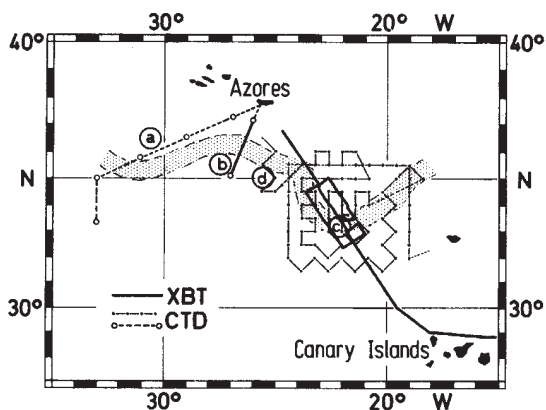


Fig. 1 Pattern of hydrographic survey performed by *FS Meteor* and *FS Poseidon* during March-April 1982. Sections a-d are referred to in Fig. 3. The dotted area indicates the estimated location of the subtropical front.

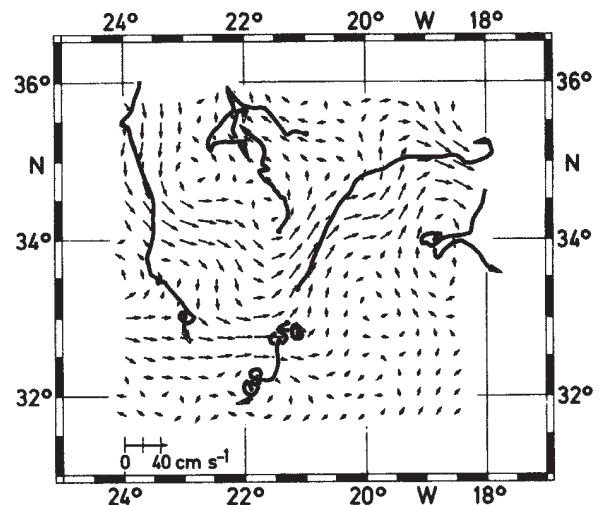


Fig. 2 Estimated geostrophic surface velocity relative to 1,500 dbar and paths of satellite-tracked drift buoys with drogues at 100 m depth. Map determined from objective analysis of geostrophic surface velocity relative to 1,500 dbar using a non-isotropic gaussian covariance function with zonal and meridional correlation scale of 100 and 65 km. Trajectories of satellite drift buoys obtained from daily fixes during *FS Poseidon* hydrographic observations.

These early data led to an easterly flow in the region where Ekman transports calculated from Bunker's¹⁰ wind data (H.-J. Isemer, personal communication) result in an Ekman convergence zone approximately along 35° N , with a seasonal meridional shift of $\pm 3^\circ$ latitude. These results on the gyre circulation and the Ekman convergence suggest a connection between the current branching from the North Atlantic Current system towards the Azores and the current related to the subtropical front in the eastern North Atlantic.

Within the framework of the Warmwassersphäre research programme at Kiel University several cruises were performed in the tropical, subtropical and moderate latitude basins of the eastern North Atlantic. Indications of frontal variability were found to exist persistently in the region between 30 and 38° N , and subsequently observational programmes were designed for 1982 cruises with the aim of resolving mesoscale features of the frontal region south and east of the Azores (Fig. 1). *FS Meteor* in March/April occupied several XBT and CTD sections including a XBT box pattern between the Canary Islands and the Azores in the northern Canary Basin and also south-west of the Azores close to Mid-Atlantic Ridge. *FS Poseidon* in April covered 82 CTD stations 30 nautical miles apart in a box pattern between the Azores and the Canary Islands. Also four satellite buoys deployed by *FS Meteor* previously were drifting in the area of the *FS Poseidon* box.

The geostrophic flow field obtained from the CTD box data is presented in Fig. 2. It reveals a current band with $\sim 60 \text{ km}$ width and 25 cm s^{-1} speed in its core, entering the box in the northwestern corner, turning east in the centre and leaving the area from the northeastern corner. This current band corresponds to large horizontal temperature gradients and also to salinity gradients in the upper ocean that specify a front. The related geostrophic shear reaches levels as deep as 650 m, more than is usually reported for surface fronts. The satellite drifters' tracks also shown in Fig. 2 conform with the general pattern of geostrophic currents obtained from the CTD data. Note that closed streamline patterns on both sides of the jet suggest the presence of mesoscale eddies with typical scales of 200 km.

The larger-scale spatial distribution of the front can be determined from further surface temperature data and temperature sections to the west of the CTD box (see Fig. 1). The surface temperatures displayed in Fig. 3 include the position of the front marked by the maximum horizontal gradient at the surface. The corresponding temperature sections are also shown

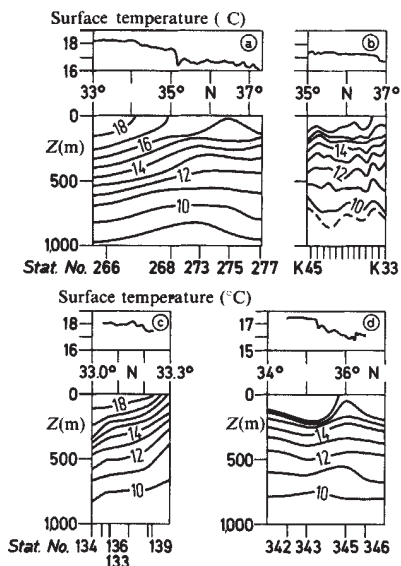


Fig. 3 Temperature obtained from sections a-d indicated in Fig. 1. Note the different scale in section c.

in Fig. 3. The front stretches zonally south of the Azores, turns to a southerly direction south-east of the Azores and in the box veers round to northerly directions. IR radiation satellite photographs confirm the existence of a continuous temperature gradient band in this general pattern (G. Hardtke, personal communication). Time variability of the front on time scales of the order of 1 month can be deduced from current meter moorings in the area. This will be discussed elsewhere.

The long-term persistence of the front can be tested by inspecting data from three different cruises: RRS *Discovery*, January 1981^{11,12}, FS *Meteor*, April 1981, and FS *Poseidon*, April 1982. The density at 250 m depth obtained in sections crossing the area under discussion is presented in Fig. 4. The horizontal density gradient related to the front is found at similar positions in all three sections. The observed surface front obviously separates regions with a permanent thermohaline mixed layer and subtropical thermocline from regions with seasonal thermocline formation, winter convection and advection of North Atlantic Current water masses. We suggest that the subtropical front found here corresponds to a branch of the North Atlantic Current that is already indicated as a broad band in Wüst's⁶ map of potential density at 200 m. This is supported by the fact that downstream volume transports in the observed baroclinic current band related to the front are of the same order of magnitude ($10^7 \text{ m}^3 \text{ s}^{-1}$) as mean meridional Sverdrup transports across this area between the Mid-Atlantic Ridge and the African coast estimated from zonal hydrographic

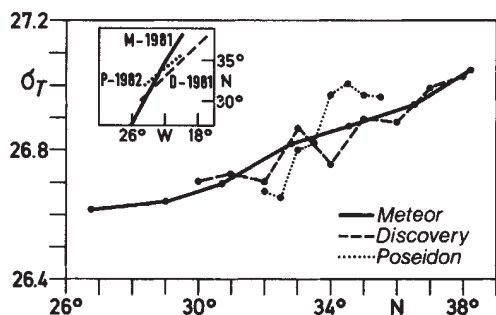


Fig. 4 Density (σ_T) at 250 m depth as function of latitude from three different cruises in the area south-east of the Azores.

sections^{11,12}. Transports in the narrow band at the front may thus constitute the major portion of the total long-term transport. It is also possible that the front observed here is a continuation of the frontal zone found¹³ to the west of this area.

The observed meandering of the front, with an even more complicated smaller-scale structure seen in IR satellite photographs, may be due to several processes. The most obvious candidates are quasi-stationary Rossby waves, which were earlier suggested¹⁴ as the cause of wave-like patterns observed in the polar boundary of the subtropical convergence in the South Atlantic, or baroclinic instability of the jet.

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Oxygen utilization rates in North Atlantic subtropical gyre and primary production in oligotrophic systems

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If the observed distribution of dissolved oxygen in deep oceans below the euphotic zone is in stationary and steady state, then it can be assumed that the *in situ* consumption of oxygen is balanced by 'ventilation', or, in other words, physical transport of oxygen from regions of higher dissolved oxygen and ultimately the sea surface. It follows, therefore, that measurement or determination of those physical transport processes, coupled with observation of the oxygen distribution will lead to an estimate of the net oxygen utilization rate (OUR). The character of such an estimate is that it is a space and time average over scales governed by the nature (space and time scales) of the transport processes, the scale of the oxygen distribution, and the climatologic nature of the system. As such, one obtains an average which is characteristically of the order of 10^6 s , 10^8 cm or more, that is large compared with scales of biological variability. I report here some new determinations of oxygen utilization rates determined by tritium dating in the North Atlantic subtropical gyre, compare them with other and previous estimates, and discuss their implications regarding primary production and nutrient cycling in an oligotrophic system.

The Beta Triangle is a triangular study area in the eastern subtropical North Atlantic whose apices are at $26.5^\circ \text{N} \times 38.5^\circ \text{W}$, $32.5^\circ \text{N} \times 30.0^\circ \text{W}$, and $22.5^\circ \text{N} \times 28.5^\circ \text{W}$. The area

was chosen as a testing ground for the Beta-Spiral calculations—a geostrophic computation which yields absolute flow velocities¹. The Triangle has sides of ~1,000 km each and is characterized by significant oxygen gradients on isopycnals. The shallow flow is of the order of 1 cm s⁻¹ to the south-west.

As part of the Beta-Spiral study, the Triangle was intensively sampled for the transient tracers tritium (³H) and ³He. The strategy was to use the observed tracer distributions to obtain an independent estimate of velocity field for comparison with the dynamical (Beta-Spiral) calculations. The preliminary results agree well (work in preparation) which lends credence to the results obtained using the tracers alone.

Tracer samples were obtained on three density levels ($\sigma_\theta = 26.45, 26.6, 26.8\%$) at 15 stations on the perimeter of the Triangle and three stations in the interior. The tritium and ³He were measured using the mass spectrometric method² to 1.0% (± 0.04 TU) and 1.6% respectively, and the ³H-³He age computed as

$$T = 1/\lambda \ln \left(1 + \frac{[{}^3\text{He}]}{[{}^3\text{H}]} \right) \quad (1)$$

where $1/\lambda$ is the tritium mean life, here taken as 17.96 yr. Age non-linearity is not significant on the time scales observed here², particularly because the tritium field is homogeneous on these levels.

The data are summarized in Fig. 1, where the regression lines obtained yield the OUR for each surface. Note that the ³H-³He age range encompassed by each surface is of the order of several years; that is, an order of magnitude greater than measurement uncertainty. The same holds true for the oxygen range. The least squares regression parameters are summarized in Table 1 with the uncertainties (1 s.d.) of the estimates. It is encouraging that the intercepts, the zero age AOU values, are not significantly different from zero.

Implicit in this analysis is the assumption that lateral mixing is small. Armi and Stommel³ have analysed the salinity fields in the Triangle and their results confirm this hypothesis. Furthermore, lateral mixing will, in the first order, be transparent as it will reduce not only the ³H-³He age gradients, but also the oxygen gradients. At least for the present analysis, therefore, it is reasonable to adopt the statistical uncertainties in the regression slopes as representative of the accuracy of this OUR determination. These are reflected in the error bars for these OUR measurements in Fig. 2.

Riley⁴ used a geostrophically 'calibrated' three-dimensional advection diffusion calculation for the North Atlantic to obtain estimates of OUR for various surfaces. Inasmuch as uncertainty in the reference level for the geostrophic calculations produces a barotropic uncertainty no more than the order of a few millimetres per second, the shallow estimates are probably accurate to within 10–20%, whereas deeper estimates may be more suspect. Those results, plotted on a depth scale, are compared with the present results in Fig. 2.

Figure 2 also shows results obtained from ³H-³He dating⁵ and from a ³H-³He calibrated lateral mixing model², both in the Sargasso Sea. These calculations differ from the Beta

Triangle OUR values in that they are 'end-point' determinations—they reference to saturation values for oxygen at zero ³H-³He ages. The Beta Triangle OUR, on the other hand, represents a gradient determination.

The results all show a remarkable consistency for depths above 1 km, particularly in light of the differences in the techniques. Although the results diverge below 1 km, this can be explained on the basis of the barotropic uncertainty in Riley's computations². Irrespective of this disparity, the bulk of the water column oxygen utilization occurs above 1 km where the results agree.

The oxygen utilization rates shown in Fig. 2 below the euphotic zone (~100 m in depth) may be integrated to yield the net oxygen consumed in the water column. The simplest approach is to use a log linear consumption rate–depth dependence, the best fit being

$$\ln \text{OUR} = -(0.68 \pm 0.17) - (0.00295 \pm 0.00027)z \quad (2)$$

where OUR is the oxygen utilization rate in ml per l per yr and z is the depth in m (positive downward). Integrating between 100 m and infinity, one obtains 5.7 mol oxygen consumed m⁻² yr⁻¹. In order that the consumption of this oxygen be supported, the water column below the euphotic zone must be supplied with ~4.5 mol C m⁻² yr⁻¹, or 55 ± 5 g C m⁻² yr⁻¹.

This corresponds to a remarkably large flux of carbon out of the euphotic zone. It is double the currently accepted mean oligotrophic primary production rate⁶, and if the level of recycling of primary production carbon approaches 80–90% as recently suggested^{7,8}, then the actual amount of carbon fixed at the ocean surface in the subtropical gyre may be several hundred grammes of C m⁻² yr⁻¹. This result, therefore, may conflict with commonly accepted ¹⁴C assimilation rate measurements of net surface primary production.

Schulenberg and Reid⁹ used observations of shallow subsurface oxygen maxima to estimate minimum surface oxygen production and hence carbon fixation rates. Their results showed carbon fixation rates 2 to 7 times higher than ¹⁴C assimilation estimates, and because that they could not account for what may be substantial photosynthetic oxygen loss to the atmosphere, represented a conservative lower limit to the disagreement between the techniques.

Primary production estimates have been based on the small bottle, long incubation technique of ¹⁴C-bicarbonate assimilation. This technique has recently been criticized by workers^{9–11} who have suggested that it may substantially underestimate the true carbon uptake. Whereas the ¹⁴C uptake technique may suffer from statistical sampling problems (due to space and time variabilities) and the possible difficulty of perturbing the systems being measured, the water mass tracer techniques achieve realistic space–time averages and are true *in situ* determinations. The results described here represent time averages extending from years to decades (in the deeper waters) and space averages from a few hundred kilometres to the whole subtropical gyre. The Schulenberg and Reid results⁹ yield time averages of a few months and space averages probably of the order of a few hundred kilometres. Clearly the water mass approach, which is based on a wide variety of techniques—transient tracers, dynamical calculations and seasonal photosynthetic oxygen cycles—presents a compelling argument for either substantially higher surface production than depicted by the ¹⁴C assimilation techniques, or less efficient recycling within the euphotic zone. Whereas the results presented here only constrain the flux out of the euphotic zone, other results^{9–11} suggest that at least part of the problem lies with the ¹⁴C techniques.

The proposed large surface productivity requires a correspondingly greater supply of nutrients (NO₃ and PO₄). The requirement is that the flux of carbon from the euphotic zone be balanced in the appropriate stoichiometric proportions by a return of nitrogen and phosphorus from the deep water. Using a transient tracer calibrated lateral transport model², one can

Table 1 AOU-³H-³He age regression results

Surface (σ_θ in ‰)	Approximate depth (m)	Intercept (ml per l)	Slope (OUR) (ml per l per yr)
26.45	200	0.10 ± 0.08	0.173 ± 0.029
26.60	300	-0.10 ± 0.11	0.196 ± 0.025
26.80	400	0.12 ± 0.18	0.169 ± 0.028

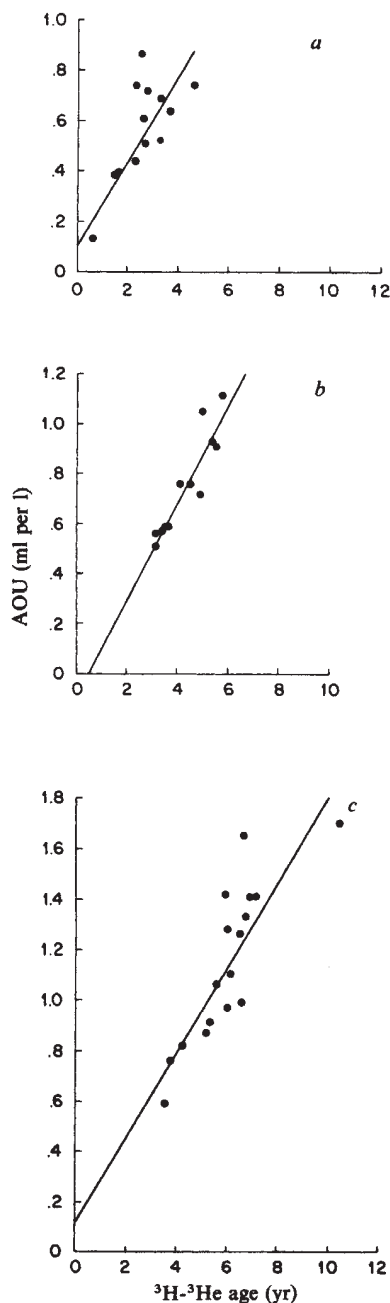


Fig. 1 AOU plotted against ^3H - ^3He age for three density surfaces *a*, $\sigma_\theta = 26.45$; *b*, $\sigma_\theta = 26.60$; *c*, $\sigma_\theta = 26.80$ in the Beta Triangle (see text).

estimate the reflux of for example, nitrate to the surface by the relation

$$F(\text{NO}_3) = \int_{100}^D \frac{C(\text{NO}_3)}{T} dz \quad (3)$$

where $F(\text{NO}_3)$ is the flux of nitrate, $C(\text{NO}_3)$ is the depth-dependent water column nitrate concentration and T is the depth-dependent residence time². The integration is from some depth D to the base of the euphotic zone (here assumed to be 100 m).

Using GEOSECS station 121 (35°59'N, 67°59'W) nitrate data¹² as a typical Sargasso Sea nitrate profile and integrating as in equation (3), one obtains a nitrate flux of 0.7 mol $\text{NO}_3 \text{ m}^{-2} \text{ yr}^{-1}$ which almost perfectly matches the requirements

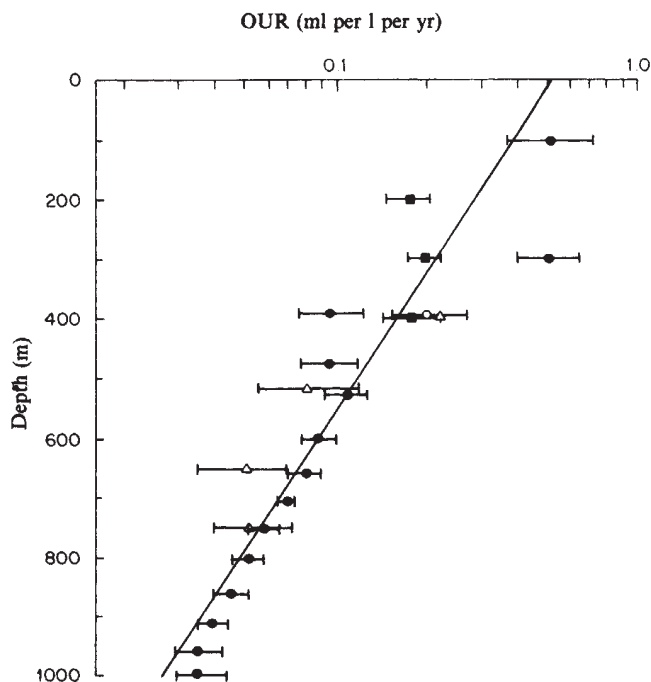


Fig. 2 Oxygen utilization rates plotted against depth from ^3H - ^3He dating (■, ○), a geostrophically calibrated three-dimensional advection-diffusion model (△) and a ^3H , ^3He calibrated isopycnal mixing model (●).

of the carbon flux reported here. A similar calculation may be performed for phosphate, but note that inasmuch as the Redfield ratios for AOU:N:P are observed quantities, and that the nutrient fluxes are 'calibrated' using the same tracer model as the OUR estimates, the nutrient fluxes must *a priori* yield a result consistent with the carbon flux.

On the basis of large scale tracer distributions, dynamical balance and subsurface seasonal photosynthetic oxygen maxima, I conclude that surface fixation of carbon in the subtropical open ocean is many times greater than deduced from ^{14}C assimilation techniques. A downwards carbon flux from the euphotic zone in excess of $50 \text{ g C m}^{-2} \text{ yr}^{-1}$ is deduced from water column OURs. These essentially independent determinations, based on different approaches are all characterized by large space and time scale averaging. This, coupled with the fact that they are independent yet yield the same results lends credence to the high rates predicted. It is therefore becoming increasingly clear that the ^{14}C assimilation techniques are in error and that oceanic 'oligotrophic' primary production is considerably higher than previously thought.

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Amino acid evidence for Devensian ice, west Gower, South Wales

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Complete inundation of South Wales by ice during the penultimate (Wolstonian?) glaciation has been widely suggested¹⁻³. Although it is generally agreed that the last glaciation was less extensive than the preceding one⁴⁻⁶, there has been controversy concerning its limits, stemming mainly from the lack of clear geomorphological evidence and the dearth of datable material⁷⁻⁹. ¹⁴C dates from the shelly till in Pembrokeshire were first thought to indicate an extension of last glaciation ice into the area¹⁰, but serious doubts were subsequently raised about the reliability of finite dates on shells of this age range^{11,12}. We present here results of amino acid analysis of shells from recently exposed till and from underlying raised beach remnants at Broughton Bay, west Gower. Total D:allo:L:iso ratios from the raised beach are consistent with analyses on shells from comparative beach remnants elsewhere in south-west Britain^{7,13-15}. Shells of 21 identifiable littoral species extracted from the till suggest inshore marine conditions similar to those at present. Amino acid ratios from *Littorina* spp. provide firm evidence for the encroachment of Devensian ice on to Gower, approximately to the limit suggested by Bowen⁷ (Fig. 1a).

Absence of unequivocal dated evidence from elsewhere in South Wales has focused attention on coastal sections of Gower, where exposures of glacial and periglacial drifts overlying prominent raised beach fragments, have been regarded as key sites for establishing the Pleistocene chronology of the area^{1,11}. Both Hoxnian⁸ and Ipswichian ages have been suggested for these fragments; however, a composite origin within the Ipswichian has been established^{7,14,16}. Bowen's limit for Devensian ice across Gower (Fig. 1a) rests largely on the

acceptance that drifts containing erratics exposed in coastal sections east of Langland Bay and near Whitford Point are *in situ* Devensian glacial deposits, whereas exposures of erratic-bearing drifts associated with the local head west of Langland Bay along the south coast are regarded as redistributed Wolstonian glacial material¹. A conjectural interglacial soil on Worm's Head¹⁷ and soils considered to be in a colluviated state along the south coast west of Langland Bay¹ have also been cited as evidence in support of this ice limit. Other workers, however, have considered that Gower remained largely ice-free during the last glaciation^{4,5} (Fig. 1a).

Recent coastal erosion at Broughton Bay (Fig. 1b) (SS: 419931) has revealed up to 5 m of a compact *in situ* diamicton containing broken and complete shells near its base (Fig. 1c). We interpret this sediment as a till; it is overlain by solifluction deposits and colluvium. The exposures run ~300 m eastwards from Twlc Point, where the till overlies fragmented raised beach sediments that contain a shell fauna comprising mainly *Littorina littoralis*. Fabric analysis (Fig. 1b) and erratic content of the till both point to a northerly (Welsh) provenance. Various marine shell species were removed from the basal 2 m of the till. The collection (Table 1) included 21 identifiable species, all of which live in the area now, except for *Arca lactea* and *Acanthocardia tuberculata*. These last two species are rare today and suggest slightly warmer conditions (N. McMillan, personal communication).

Table 1 Mollusca from Broughton Bay, South Wales

Gastropods	Bivalves
<i>Patella vulgata</i>	<i>Arca lactea</i>
<i>Gibbula magus</i>	<i>Mytilus edulis</i>
<i>Littorina saxatilis</i>	<i>Ostrea edulis</i>
<i>Littorina littoralis</i>	<i>Chlamys varia</i>
<i>Turritella communis</i> Risso	<i>Arctica islandica</i>
<i>Nucella lapillus</i>	<i>Acanthocardia tuberculata</i>
<i>Ocenebra erinacea</i>	<i>Cerastoderma edule</i>
<i>Nassarius reticulatus</i>	<i>Venus striatula</i>
<i>Buccinum undatum</i>	<i>Venerupis rhomboides</i>
	<i>Venerupis pullastra</i>
	<i>Macoma balthica</i>
	<i>Corbula gibba</i>

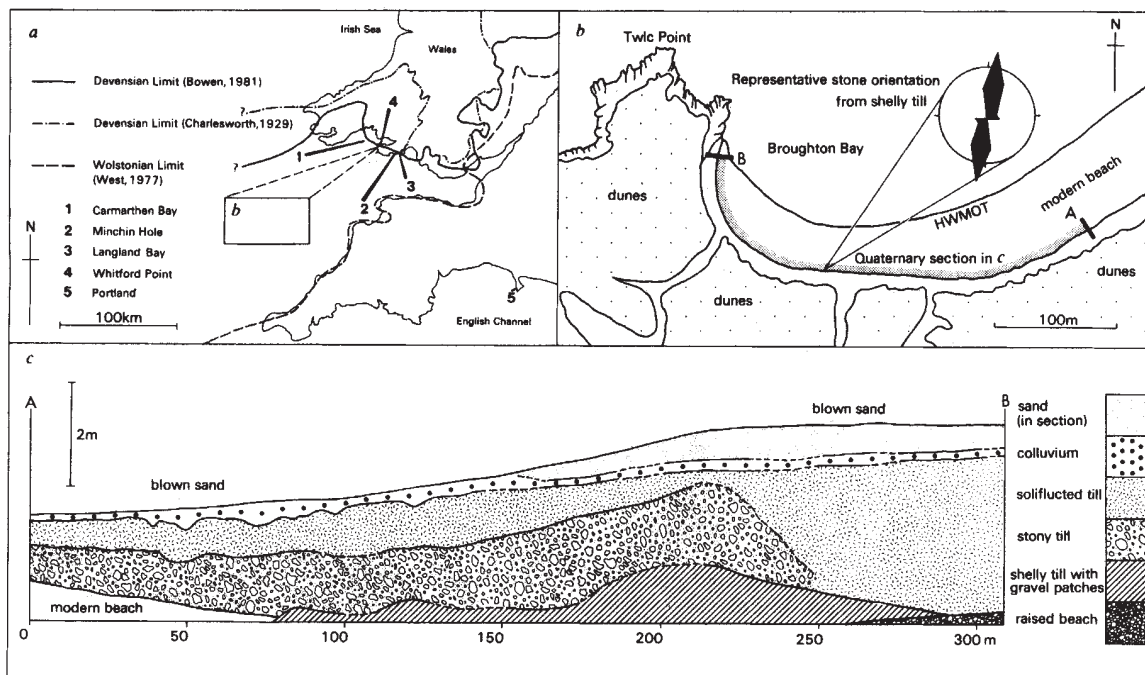


Fig. 1 South Wales. a, Site locations and suggested ice limits: —, Devensian limit⁷; - - -, Devensian limit⁴; ---, Wolstonian limit²; b, site plan; c, generalized Quaternary stratigraphy, Broughton Bay.

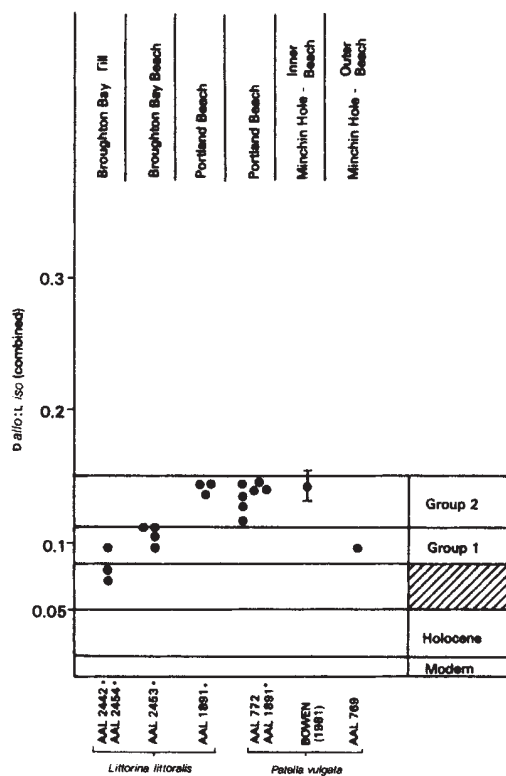


Fig. 2 Comparative amino acid ratios for south-west Britain (after ref. 14). * New data from the present results.

Littorina littoralis shells from the till and raised beach were prepared for amino acid analysis by the method described elsewhere^{14,15}, and the results are shown in Table 2. The tight clustering of the total ratios for these *Littorina* shells from the raised beach (AAL-2453) of 0.107 ± 0.014 suggests strongly a non-mixed population. These values are lower than those obtained for *L. littoralis* from the raised beach at Portland (0.137 ± 0.005) ($n = 3$, AAL-1891, A, B, C). (The ratios reported from Broughton Bay have been recalibrated against other samples run earlier. This procedure was necessary because detailed analysis involving many tens of runs indicated that the preparation method used for about 9 months in 1981 gave ratios that were consistently too high¹⁸.) *Patella vulgata* from the same unit at Portland, collected by Briggs, gave total *D allo:L iso* ratios of 0.13 ± 0.005 (AAL-1891, D, E, F). Comparable results derived from the 8 m beach on Jersey have been related to an absolute U-series date of 121 ± 12 kyr BP (ref. 13), and an average amino acid ratio of 0.14 for *P. vulgata* from the inner raised beach at Minchin Hole gives further indication of comparable relative ages for some of the raised beach fragments in south-west Britain⁷. The Broughton Bay ratios fall into group 1 of Andrews *et al.* (Fig. 2) and are thus probably younger than 121 ± 12 kyr BP.

The validity of these correlations rests principally on the acceptance of a comparable inter-site temperature history and of a similar kinetic relationship between the species *P. vulgata* and *L. littoralis*. The present mean annual temperatures of 10.9°C at Gower (Swansea), 10.8°C at Portland and 11.9°C at Jersey (St Helier) are reasonably close. However, whether temperatures at these sites have consistently remained as close throughout the past 100 kyr, particularly during cold phases, is less certain. Taxon-dependent racemization differences are not judged to be a factor here on the basis of the close comparison between *L. littoralis* and *P. vulgata* (Fig. 2). Consequently, a correlation of the Broughton Bay raised beach with other beach fragments broadly regarded as Ipswichian (stage 5) in south-west Britain seems justified.

Table 2 Amino acid racemization results

Sample		Species	D allo : L iso (combined)	
AAL-2453	a	<i>Littorina littoralis</i>	0.115	Beach
	b	<i>Littorina littoralis</i>	0.107	
	c	<i>Littorina littoralis</i>	0.098	
	d	<i>Littorina littoralis</i>	0.115	
AAL-2442	a	<i>Littorina littoralis</i>	0.082	Till
	b	<i>Littorina littoralis</i>	0.073	
AAL-2454	a	<i>Littorina littoralis</i>	0.098	
Average D allo : L iso (combined):				
<i>Littorina littoralis</i> (Broughton Bay beach)			0.107 ± 0.014	
<i>Littorina littoralis</i> (Broughton Bay till)			0.082 ± 0.022	
<i>Littorina littoralis</i> (Portland beach)			0.137 ± 0.005	
<i>Patella vulgata</i> (Portland beach)			0.130 ± 0.005	

Littorina littoralis from the till yielded a slightly lower mean total *D allo:L iso* value of 0.082 ± 0.022 than that from the raised beach (0.107 ± 0.014) but still showed a relatively unmixed population. Hence, whereas the raised beach at Broughton Bay can be correlated by amino acid ratios with the outer beach at Minchin Hole, ratios for the till are on average slightly younger, although the ratios overlap at the 95% confidence level. A maximum relative age for the ice advance would be indicated by the ratio of the least racemized *L. littoralis* shell, namely 0.073 ± 0.007 .

The amino acid evidence therefore indicates that the shells in the till date from no earlier than the Ipswichian ($\sim 121 \pm 12$ kyr BP (ref. 13)), and that some shells post-date the raised beach event at Broughton Bay. This evidence strongly suggests, therefore, that it must have been ice of Devensian age, and no older, which moving southwards across the eastern part of Carmarthen Bay, incorporated shell litter that had accumulated during the preceding interglacial. The fabric and erratic content of the shelly till deposited at Broughton Bay support this interpretation.

The point at which the Broughton Bay shelly till was deposited within the Devensian period cannot be resolved easily. The amino acid ratios themselves indicate the shell fauna is most probably early Devensian in age. This, however, only establishes a maximum age for a glacial event which incorporated the shells in the diamict. On the other hand, stratigraphical^{1,11} and palynological^{19,20} data from the local area, point strongly to last glaciation deposits in the vicinity being late Devensian in age. The lack of amino acid ratios extending into middle or late Devensian times does not necessarily contradict the latter view; with sea level falling at the close of the Ipswichian, much of Carmarthen Bay would have remained as dry land during this period, preventing further accumulation of younger marine shells. Thus whilst the amino acid ratios presented above do not allow precise dating of the Broughton Bay shelly till within the Devensian period, they do demonstrate the encroachment of Devensian ice sheets on to west Gower. Furthermore, our results demonstrate the potential of amino acid ratios for resolving certain chronological aspects of the various shelly drifts of the UK and elsewhere²¹.

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Comparison between the hot spot and geomagnetic field reference frames

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Hot spots are located on the Earth's surface and are caused by hot plumes of upwelling mantle material. They manifest themselves by volcanic activity which changes its location in response to the movement of the lithosphere over the mantle plume. It has been suggested^{1–3} that hot spots are relatively stationary with respect to each other, and also that they represent a frame of reference with which to measure the absolute movement of the lithospheric plates over the surface of the Earth. Another frame of reference can be partially defined by the position of the mean magnetic dipole field of the Earth. As the dipolar field is believed to be closely related to the spin axis, this frame of reference automatically gives information about the palaeolatitude and orientation of the lithospheric plates. But since the position of the ancient dipole field of the Earth does not give any information about palaeolongitude, and since in many cases the definition of the field is relatively poor due to less than adequate rock collections during the time of interest, hot spot reference frames have been favoured by many people. We now report a comparison of the two frames of reference and show that over the past 200 Myr there has been relative movement between the two frames of reference of about 20°, and that this motion has not been constant with time.

We have developed a new method for calculating palaeomagnetic poles for individual continents⁴. As the relative positions of most continents are moderately well known during the Neozoic, it is also possible to use the palaeomagnetic information from one continent to define with greater accuracy the palaeomagnetic pole for another continent. Data for North America are shown in Table 1. The apparent polar wandering (APW) path shown on the left is that generated solely from North American palaeomagnetic data⁴. The APW path on the right has been generated using data from five continents (North America, South America, Africa, Europe and Australia) and using the relative rotation parameters given by Barron *et al.*⁵ to express the palaeomagnetic poles of all continents in the North America frame of reference. The method of analysis of the rotated poles is similar to that described in ref. 4. There are two important results obtained by using the world data to define a North American polar wandering path.

First, the accuracy with which the pole may be defined can be significantly increased by using data from the other con-

tinents as well. The average value of α_{95} (the radius of the 95% circle of confidence around the mean) has decreased from 5.7° to 3.5° by using the data from Australia, South America, Europe and Africa in addition to those from North America, to define the North American APW path. Second, the two polar paths can differ by almost 10°, which occurs during the Upper Cretaceous and the Middle and Upper Jurassic. The ratio between the average values of α_{95} using just the North American data and using the data from all five continents is 0.61. If the relative positions of the continents were perfectly well known during the time interval considered, there should have been a reduction in the value of α_{95} equivalent to the square root of the ratio of information contained in the North American data to the information contained in the data from all five continents, which is 0.53. Thus, although the relative position information for the continents is not perfect, the reduction in α_{95} approaches that which would be expected considering the added information content and an assumption that the relative position information is correct. This is obviously not true in some cases; for instance the 200 Myr information shows no reduction in α_{95} whereas it should have been reduced from 2.8° to 1.6° if it were purely dependent on the amount of information used.

We compare the finite motions predicted by hot spots, and APW paths back to 200 Myr. Morgan¹ has defined the motions of the major continental plates with respect to a hot spot reference frame for this time interval. It is possible to use these data to generate pole positions on the assumption that the hot spot reference frame is fixed with respect to the geomagnetic field. We can then compare these calculated polar positions with those generated using palaeomagnetic data to see if the assumption holds true. We show three sets of results. The first set is given in Table 2 and compares the two polar wandering paths for Europe. The last column of this table shows the angular deviation between the two sets of results. Deviations increase from the present back to 50 Myr at which time there is a reduction in the deviation. From 70 to 180 Myr the deviation again increases and reaches a value of 17.6°.

When we generate polar wandering information for any continent we use relative position information which is different from that used by Morgan. Therefore, we obtain different results for different continents. This set of results (which

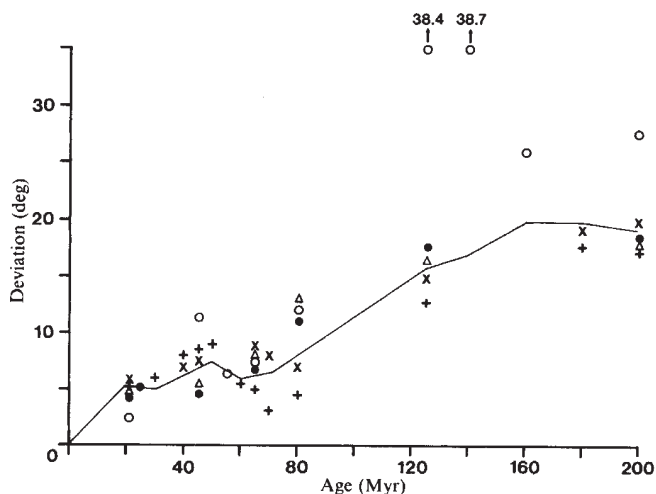


Fig. 1 Deviations between APW paths predicted using a hot spot reference frame and those using palaeomagnetic data. The symbols compare palaeomagnetic data in which relative positions between the continents used to combine the palaeomagnetic data are those given in ref. 5 with the hot spot data given in ref. 1. The line shows a comparison in which the relative positions of the continents used to combine the palaeomagnetic data are those given in ref. 1, compared with the same hot spot data. ○, Australia; +, Europe; △, Africa; ●, South America; ×, North America.

Table 1 Apparent polar wandering path for North America

Age	Using North American data			Using data from five continents			Deviation (deg)
	Lat. (°N)	Long. (°E)	α_{95} (deg)	Lat. (°N)	Long. (°E)	α_{95} (deg)	
20	85.9	151.1	3.6	84.7	167.5	2.2	1.8
30	84.7	157.8	3.6	83.0	162.0	2.4	1.8
40	83.3	165.4	3.5	81.1	168.5	2.5	2.2
50	83.1	178.2	3.5	79.7	177.3	2.5	3.4
60	80.1	-176.2	4.7	78.2	178.7	3.0	2.1
70	76.7	-171.6	5.7	77.6	-178.3	3.6	1.7
80	70.6	-164.7	7.6	77.6	-176.6	4.3	7.7
90	68.8	-167.9	8.2	78.1	-179.4	4.1	9.8
100	69.1	-174.0	8.1	76.9	-174.6	3.7	7.8
110	69.5	-177.9	6.7	73.5	-168.0	3.5	5.1
120	67.9	179.1	6.3	71.9	-170.5	3.7	5.3
130	66.2	164.3	5.8	71.4	173.3	4.0	6.1
140	66.0	156.5	5.6	72.2	157.5	4.3	6.2
150	66.6	147.8	5.7	73.8	137.4	4.4	8.0
160	69.7	132.3	7.5	76.2	106.4	4.6	9.8
170	75.9	121.3	9.9	75.5	89.6	4.4	7.7
180	69.9	96.6	5.4	72.2	90.3	3.5	3.1
190	66.2	93.9	3.5	69.8	94.1	3.1	3.6
200	63.6	94.1	2.8	68.4	94.7	2.8	4.8
Average			5.7			3.5	

includes the values for the last column in Table 2) is presented in Fig. 1, in which the scatter for any one age reflects the fact that our relative position information is different from that given by Morgan. In particular, the very large deviations seen for Australia between 125 and 140 Myr are due to the fact that Morgan's position for Australia during these times is such that the APW path for Australia would predict poles very close to the present pole, whereas in our reconstruction Australia lies considerably further south and is rotated compared with its present position. It should be possible to use palaeomagnetic data to determine which of these reconstructions is better, but unfortunately, there are almost no data for Australia during this time interval⁶. The points plotted in Fig. 1 suggest an increase in the deviation between the present and about 40 Myr before which there is a suggestion of a slight decrease in the deviation. The deviation increases between about 70 and 180 Myr. The close grouping of the data from the four continents surrounding the Atlantic Ocean at 200 Myr is because our reconstruction of Pangaea⁷ is very similar to that of Morgan¹, the only major difference relevant to this study being in the relative position of Australia.

Duncan⁸ has also produced absolute motion parameters of the southern continents with respect to a hot spot frame of

reference, back to 100 Myr ago. Deviations calculated using his data fall within the scatter of the points shown in Fig. 1.

We have also generated another global polar wandering path using the same palaeomagnetic data but using the relative position information given by Morgan¹. The polar wandering path for North America is in fact very similar to that given in Table 1. Deviations between the two average 2.5°, the largest being 6.2° at a time of 160 Myr. The inaccuracy for each pole is marginally greater for Morgan's reconstruction data (mean $\alpha_{95} = 3.6^\circ$). Comparison between this polar path and the hot spot data need be done only once, since the same relative position information was used for both sets of data. The deviation between the two is shown by the line in Fig. 1.

The average motion between the two frames of reference during the 200 Myr interval of observations is about 19°, representing a speed of motion of 10.6 mm yr⁻¹. The relative speed of motion was faster during the period 60–160 Myr, being about 15.6 mm yr⁻¹. The motion established by the point at 21 Myr gives a speed of motion between the two reference frames of 28.0 mm yr⁻¹, but the error in this number is much greater because it is based on a smaller deviation.

A word must be said about errors. Presumably, the hot spot traces, being marked by geological features, are accurately placed to within 1°. There may, however, be errors associated with assigning ages to the traces. Because most of the hot spots used by Morgan are within continental regions at the start of his reconstruction, the ages should be much better known than for oceanic hot spot traces. A typical radiometric age has an error of 3% of the total age, or 6 Myr in the case of the 200 Myr hot spot traces. At a motion of 30 km Myr⁻¹, this age error is equivalent to an angular error of about 1.6°. Thus a possible hot spot error might be given by the square root of the sum of the squares of 1.6 and 1, or 1.9°. The polar error could likewise be considered to be made up of errors due to the palaeomagnetism (average of 3.5°, Table 1) and errors due to plate reconstruction (taken as the mean polar difference between Morgan's relative position data and ours of 2.5°) giving a total palaeomagnetic polar error of 4.3°. The total possible error in the deviation will be in the region of 4.7°, and so the observed deviation of over 19° observed at 200 Myr represents a real effect. Possible errors in the reconstruction of Gondwanaland will have little effect on the overall result since only 7% of the palaeomagnetic data come from Australia. The remaining 93% of the data come from Europe, North and South America, and Africa, whose relative positions are well known apart from minor differences between various models.

Morgan¹ also showed that there was relative motion between his hot spot reference frame and the geomagnetic field. But the greater number of palaeomagnetic data that we have used has described the effect more accurately. The details of the relative motion revealed by this study also differ from those suggested by Morgan. This relative motion between the hot spot and the geomagnetic field reference frames is considerably greater than the relative motions which take place between the hot spots which are about 5° in 200 Myr according to Morgan. If this supposition is correct, it is necessary for the layer within the mantle at which the hot spots are generated to be drifting slowly with respect to the spin axis of the Earth, to which we assume the geomagnetic field is fixed.

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Table 2 Comparison between observed APW path for Europe and that predicted by motion of Europe w.r.t. the hot spots

Age	Predicted APW position		Observed APW position*		Deviation (deg)
	Lat. (°N)	Long. (°E)	Lat. (°N)	Long. (°E)	
21	87.7	61.2	85.1	150.3	5.4
30	86.7	79.8	83.5	144.3	5.9
40	85.6	77.8	82.0	150.5	7.9
45	84.8	79.8	81.7	153.4	8.5
50	84.4	81.5	81.3	156.3	9.0
60	84.3	134.1	80.5	165.6	5.5
65	83.6	143.7	80.5	172.3	4.9
70	81.0	159.9	80.5	178.9	3.1
80	75.9	184.7	80.3	189.8	4.5
125	67.3	170.9	73.6	204.3	12.6
180	52.7	116.6	70.2	113.5	17.6
200	49.5	110.4	66.6	118.6	17.6

* Observed APW position calculated using data from all five continents plus the relative position information.

Thermal convection in magmas generated by hot-plate heating

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A condition is derived here for the onset of thermal convection in a layer of silicate partial melt that is generated by suddenly and continuously supplying an anomalous heat flux or by maintaining an anomalous high temperature on a plane at some depth within the Earth so that the overlying material is heated to greater-than-solidus temperatures. The results show that for highly fluid basaltic magmas convection may begin in a layer only 10-m thick, whereas for viscous rhyolitic magmas, at least a 10-km thick layer is required. The fact that thermal convection may occur within such a thin layer of basaltic partial melt may partially explain the homogeneity of large volumes of flood basalts that occur. Since convection within rhyolitic partial melts is unlikely, homogeneous rhyolites must be the result of mixing in magma chambers.

Although the hot-plate model is an oversimplification of the process of melt generation within the Earth, it may be relevant to the generation of rhyolite magmas near subduction zones, where mafic intrusions may supply heat to melt partially the overlying crust. Hodge¹ has suggested such a model for the generation of granitic batholiths. Hodge's calculations show, for example, that the heat transferred from a cooling mafic sill 4 km thick, initially at 1,200 °C, that is emplaced in country rock initially at 500 °C and which has a melting temperature of 700 °C, is sufficient to generate nearly a 1-km thick layer of partial melt of granitic composition within 40,000 yr. Hodge did not discuss whether convection could occur within the partial melt layer, however.

The nature of the heat source that would act as a hot-plate for the generation of basaltic magma in the mantle cannot be specified with certainty. Yoder² has suggested that a peridotite diapir that is depleted of its basaltic constituents or that an inflection in the geotherm might provide the required heat flux.

Although the hot-plate model is an approximation to natural mechanisms of magma generation, the model is a useful first step towards an understanding of magma genesis and transport. Moreover, the hot-plate process is amenable to laboratory investigation. Yoder³ is carrying out experiments on the onset of convection in a two-component silicate partial melt heated from below. The present calculation may provide a useful tie between theory and experiment.

A simplified schematic of the hot-plate model is shown in Fig. 1. In reality the partial melt region is very complicated. In a multi-component system, the partial melt fraction and its compositions are a function of temperature, pressure, and bulk composition of the source rocks; consequently, the physical parameters of the system will vary as a function of height in the partial melt layer. It is not possible to take into account changes in density, specific heat, thermal conductivity, viscosity, permeability, and so on; so the conditions for the onset of thermal convection in a growing layer of silicate partial melt will be determined by assuming a system with uniform properties and by assuming melting at a fixed temperature.

Observations on the partial melting of silicate rocks in laboratory experiments have shown that when partial melts form, the liquid coats the grain boundaries of the remaining solid matrix^{4,5}. The melt thus provides porosity and permeability within the partial melt, and the magma may flow along grain boundaries. The dynamics of the magma will be assumed to be governed by Darcy's law for flow in a porous medium⁶⁻⁹.

The problem is to determine the condition for the onset of convection in a fluid-saturated porous medium heated from below, where the height of the layer grows with time in a

manner determined by the rate of advance of solidus boundary. When the layer reaches a certain critical thickness, the conduction regime will become unstable and thermal convection will be set up. The condition for the onset of convection is expressed by a dimensionless parameter termed the Rayleigh number, the exact form of which depends on the boundary conditions that are applied¹⁰. The bounding surfaces are assumed here to be rigid and the temperature at the top is the melting temperature T_m , and at the base of the layer a fixed temperature T_1 will be maintained. With these boundary conditions, the critical Rayleigh number, R , is $4\pi^2$ (ref. 10). Thus:

$$R = \frac{\rho_t s \alpha g X(t)^2 K (\partial T_0 / \partial x)}{\lambda \nu} \geq 4\pi^2 \quad (1)$$

where ρ_t , s , and α are the density, specific heat, and thermal expansion coefficient of the melt, respectively; g is the acceleration due to gravity; $X(t)$ the thickness of the partial melt layer; K the permeability; $(\partial T_0 / \partial x)$ the mean conductive temperature gradient maintained across the layer, λ the thermal conductivity of the solid-fluid mixture, and ν the kinematic viscosity of the melt.

The mean conductive gradient is found from a solution to the Stefan problem¹¹:

$$T_0(x, t) = T_m + \Delta T \{1 - [\operatorname{erf} x / 2(at)^{1/2}] / \operatorname{erf} Q\} \quad (2)$$

where $\Delta T = T_1 - T_m$, $a = \rho_t s / \lambda$, and Q is found from the relationship

$$Q (\operatorname{erf} Q) \exp(Q^2) = St / \sqrt{\pi} \quad (3)$$

where the Stefan number, St , is defined as $St = s \Delta T / \chi L$; χ is the partial melt fraction and L is latent heat of melting. The motion of the phase boundary is given by:

$$X(t) = 2Q(at)^{1/2} \quad (4)$$

From equation (2), the mean temperature gradient is given by $\Delta T / X(t)$.

The interconnected pathways that provide the permeability within the partial melt zone are undoubtedly quite complex. Nevertheless, a reasonable approximation to the permeability can be obtained by assuming that the solid matrix is cubical with average grain diameter b , and that the melt fills the interconnected channels of width d . It is assumed that there are no isolated pockets of partial melt. The porosity, which can then be defined as the degree of partial melt, χ , is given by⁸ $\chi = 3\pi d^2 / 4b^2$ and the permeability by¹² $K = \chi d^2 / 96$. Eliminating d gives $K = \chi^2 b^2 / 72\pi$. On substituting for K and $(\partial T_0 / \partial x)$ into equation (1) gives the condition:

$$X(t) \geq \frac{288\pi^3 a \nu s}{\alpha g \chi^3 b^2 L St} \quad (5)$$

Yoder³ has suggested that 30% partial melt of basaltic composition is generated at the invariant point in the eutectic-like

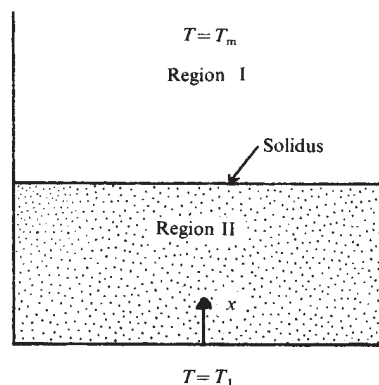


Fig. 1 Schematic of hot-plate heating model. Region I is solid at the melting temperature T_m . Region II is partially molten. A fixed temperature $T_1 > T_m$ is maintained at $x = 0$.

Table 1 Values of parameters considered

Parameter	Value	Comments
ν	$10-10^6 \text{ cm}^2 \text{ s}^{-1}$	See text.
a	$10^{-2} \text{ cm}^2 \text{ s}^{-1}$	Typical igneous rock values.
s	$0.25 \text{ cal g}^{-1} \text{ }^\circ\text{C}^{-1}$	
α	$3 \times 10^{-5} \text{ }^\circ\text{C}^{-1}$	
g	10^3 cm s^{-2}	See text.
χ	0.3	
b	$10^{-1}-1 \text{ cm}$	See text.
L	135 cal g^{-1}	
St	0.2-2.0	Corresponds to a temperature range of 32-320 °C across the partial melt layer.

melting of garnet-peridotite and that the degree of partial melt increases with temperature. The degree of melt for rhyolites depends on the bulk composition, among other factors. Some rhyolite compositions, minimum melt rhyolites, form nearly 100% melt at a fixed temperature³. A conservative average value of $\chi = 0.3$ is assumed. The assumption of a fixed melting temperature T_m in this case means that 30% of the material melts at T_m ; the rest of the material remains solid until T is considerably greater than T_1 .

The latent heat of fusion is taken to be 135 cal g^{-1} , the value given by Yoder¹³ for garnet-peridotite. The latent heat for rhyolite may be lower. Table 1 lists the numerical values of all the parameters used to evaluate equation (5). Substitution of the values gives

$$X(t) = 2 \times 10^2 \nu / b^2 St \quad (6)$$

where ν , b are given in c.g.s. units.

Figure 2 gives a plot of equation (6) as a function of ν for a few representative values of b and St . The results show that for low viscosity, which is characteristic of basaltic magmas¹⁴, and coarse-grained source rocks, that convection will begin in a layer of partial melt as little as 10-m thick. With smaller values of the grain size or Stefan number, or larger values of viscosity, convection will set up in a layer of 1-km thickness or less. From equations (3) and (4), the time required to generate a 1-km thick layer, when $St = 2$, is $\sim 10^4$ yr, which is not an unreasonable time for a thermal event. From these calculations it would seem that convection will probably occur in the partial melt zone, and thus homogenize the magma before its leaving the source zone. This effect may partially explain the large volumes of nearly homogeneous basaltic magma that have been episodically extruded throughout geological history.

On the other hand, if the viscosity is of the order of $10^4-10^6 \text{ cm}^2 \text{ s}^{-1}$, as may be expected for rhyolitic magmas¹⁵, and the other parameters are held fixed as in Table 1, the thickness of the partial melt layer must exceed 10 km before convection will begin, even for the most ideal case shown in Fig. 2. For this case, the time required for generation of the layer must exceed 10^6 yr. These values of layer thickness and time required for convection to begin in a rhyolitic partial melt are not totally

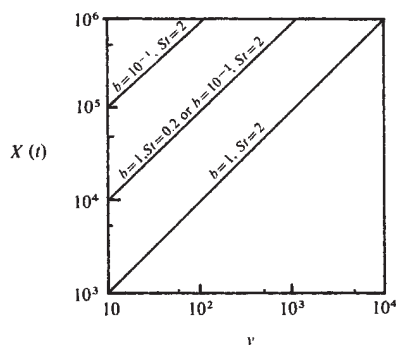


Fig. 2 Thickness $X(t)$ of partially melt layer at initiation of thermal convection as a function of viscosity ν for typical values of grain size b and Stefan number St . All dimensions are c.g.s.

out of line, but they must be viewed as lower bounds. If, for example, $b = 10^{-1} \text{ cm}$ or $\nu = 10^6 \text{ cm}^2 \text{ s}^{-1}$ and the other values are held constant, $X(t) = 10^3 \text{ km}$. This thickness is clearly unrealistic. It is not likely that modifications in the other parameters will alter this result much. In particular, for the porous medium model to hold χ must be ≤ 0.5 . For a greater value than this, the solid matrix structure will collapse¹⁶. Moreover, Ahern and Turcotte⁸ have shown that the matrix will begin to collapse at the bottom of the partial melt layer as a result of differential stresses, when the thickness of the layer is of the order of 100 m. Finally, if a rhyolitic partial melt layer extends to fairly shallow depths, brittle fracture may occur above the layer and the magma may escape before being mixed by convection within the source zone. Thus if rhyolitic magmas are homogenized at any time, the convection process must occur in subsidiary magma chambers.

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Pattern of opening rates along the axis of the Mid-Atlantic Ridge

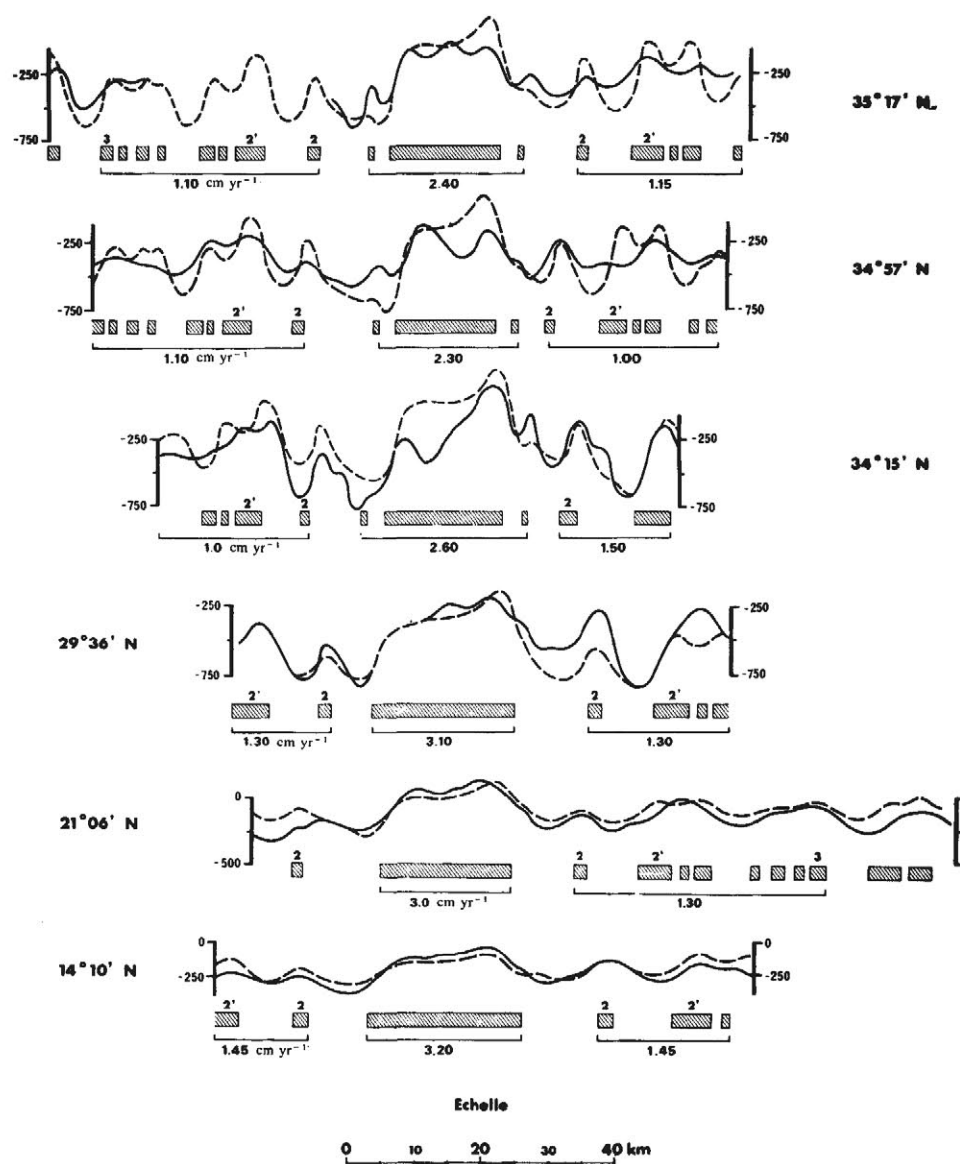
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Vine and Wilson¹, using the Vine and Matthews² hypothesis, were the first to try to relate the magnetic pattern over the crests of the mid-ocean ridges to the known geomagnetic time scale to determine the spreading rate over the past few million years. Many determinations of spreading rate at the axis of the ridge during Plio-Pleistocene times have been published: Le Pichon³ and Morgan⁴, for example, used spreading rates computed for the axial magnetic pattern (10 Myr: anomaly 5 of Heirtzler *et al.*⁵ or for the past 5 Myr respectively) even when trying to assess the properties of instantaneous plate kinematics. Likewise in the systematic inversion of Minster *et al.*⁶ anomalies 3 and 5 were generally used thus averaging plate speeds over the past 5-10 Myr. Minster and Jordan⁷ sought to improve this and redetermined the spreading rates using magnetic anomalies 2 and 2' yielding a mean averaging interval of <3 Myr. Here we examine plate behaviour over a shorter time scale in the reasonably well-surveyed axial region of the Mid-Atlantic Ridge south of the Azores. The average opening rates we obtained for this region for magnetic anomalies 1 (Brunhes), 2 and 2' using two different geomagnetic time scales indicate that (1) there was a relative slowing down of the opening between anomalies 1 and 2 which may be of global significance; (2) the opening is compatible with a rigid plate model when it is averaged over ~ 2.5 Myr although for shorter averaging times (1.8 and 0.7 Myr) there is some evidence of non-rigidity of the plates. Thus different segments of the Mid-Atlantic Ridge may display an independent behaviour and, with respect to relatively short time intervals, may show departures from characteristics typically associated with slow accretion.

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Fig. 1 Magnetic anomaly profiles along R/V *Jean Charcot* tracks during the *Vema* cruise (solid lines) at six locations along the Mid-Atlantic Ridge south of the Azores. IGRF 75²⁶ has been removed from the total intensity data. The synthetic anomaly profiles (dotted lines) have been constructed piecemeal from the distribution of a uniformly magnetized horizontal magnetic source layer shown in hatched (positive polarity) and white (negative) below each profile pair. The depth to the top of the source layer is, from north to south, 2,000–2,300–2,800–3,100 and 2,500 m. This corresponds to the average ocean depth along each profile. The azimuth of the observed magnetic profiles is, from north to south, 94–101–100–107–104 and 90°. The profiles are approximately normal to the local strike of the ridge. Modelling includes the Jaramillo event for the three northern profiles. For the other profiles the axial rate applies only to the Brunhes block and Jaramillo is not resolved.



Six R/V *Jean Charcot* magnetic profiles approximately normal to the strike of the Mid-Atlantic Ridge were acquired during the 1977 *Vema* cruise in conjunction with multi narrow-beam bathymetry obtained with the Seabeam system^{8,9}. The Seabeam bathymetry demonstrates that each profile is within one given crustal block bounded by fracture zones. The magnetic anomaly profiles distributed between 14°10' N and 35°17' N are presented in Fig. 1 with the corresponding synthetic profiles computed from the magnetic source distribution shown below each profile pair. The magnetic modelling used to compute synthetic profiles is quite simple: in each case we have assumed an horizontal magnetic source layer of uniform thickness (400 m) and intensity (0.0024 e.m.u. for all profiles except for the profile at 34°57' N where the magnetization has been chosen as 0.02 e.m.u.). We also assume accretion to take place on a line of zero width. The uniform depth of each source layer has been chosen to match the average ocean depth along each *Jean Charcot* profile to take into account the varying distance to the magnetic source layer due to topographic variations along the strike of the Mid-Atlantic Ridge^{10,11}.

Synthetic anomaly profiles were generated at several rates using the Talwani *et al.*¹² time scale and the match between observed and synthetic anomalies was done piecemeal. Thus the synthetic profiles shown in Fig. 1 are composite. The result-

ing magnetic source distribution enables one to pick reversal transitions. Modelling with more advanced inverse methods¹³ gives the same results for the reversal transitions as the direct modelling used here. The effect of variable layer thickness on reversal position can be shown to be very small (a few hundred metres at most).

Average opening rates can be calculated for different time intervals. We have chosen a rate corresponding to addition of new crust during the Brunhes epoch (anomaly 1; 0.69 Myr) and the average rates since the times (1.78 and 2.635 Myr) corresponding to the middle of anomalies 2 and 2' respectively according to the time scale of Talwani *et al.*¹². The observed opening rates averaged over these three different time intervals are plotted as a function of latitude in Fig. 2a which also shows the data obtained in the Famous area around 36°58' N by Greenwalt and Taylor¹⁴, Needham and Francheteau¹⁵, Renard¹⁶ and MacDonald¹⁷. The opening rate data are compared with the pattern of opening predicted by the inversion model of Minster and Jordan⁷ for the Mid-Atlantic Ridge south of the Azores using bounds derived from the inversion model uncertainties. We compare opening rates computed along the ship's profiles which are nearly normal to the local strike of the ridge crest on the basis of Seabeam data with opening rates calculated along the direction of relative motion of the plates.

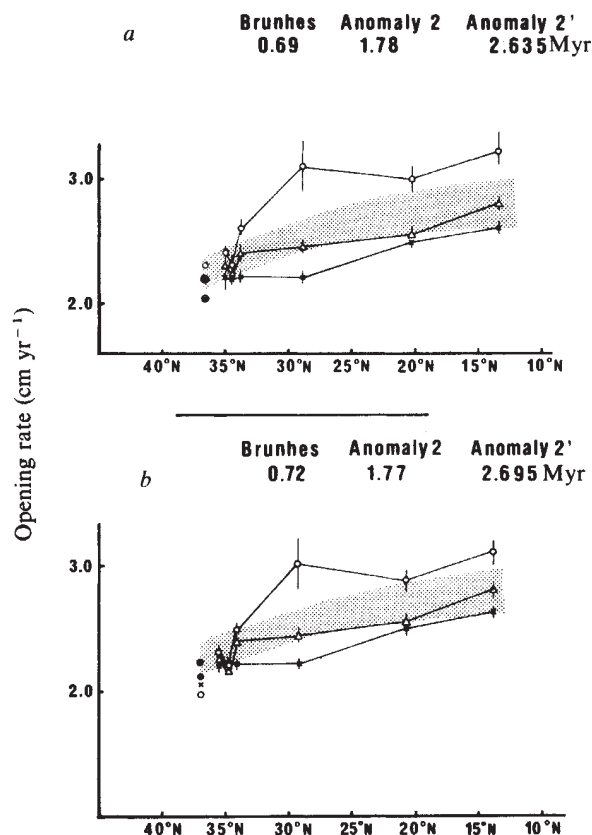


Fig. 2 Along-strike distribution of spreading rates. The total opening rates have been calculated for the Brunhes epoch ($\circ$), for a time span extending to the middle of anomaly 2 ($\times$) and for a time span extending to the middle of anomaly 2' (Δ). Error bars for spreading rates have been estimated from a comparison between the synthetic model and the observed profiles. Three rates have been estimated for the latitude of the FAMOUS area at $36^{\circ}58'N$: a rate of 2.04 calculated for anomaly 1 and 2 times by MacDonald¹⁷; a rate of 2.2 for anomaly 1 time¹⁵, up to anomaly 2 (ref. 27) and up to anomaly 3¹⁶; a rate of 2.3 obtained by Greenewalt and Taylor¹⁴ for anomaly 1 time. Two geomagnetic time scales were used (Fig. 2a, Talwani *et al.*¹²; Fig. 2b, Mankinen and Dalrymple¹⁸). The shaded band represents the opening rates computed from the pole of Minster and Jordan⁷ at $80.43^{\circ}N$ – $56.36^{\circ}W$ (angular rotation rate $0.258^{\circ}Myr^{-1}$) and includes the effect of the oval of confidence in pole position computed by these authors.

The difference between the two directions is always $<10^{\circ}$ and thus the correction factor never exceeds 0.984. The pattern of observed rates falls into the permissible band only when the averaging is conducted over a time interval of ~ 2.6 Myr. This is also true, although less so, when the data are averaged over 1.78 Myr. It is apparent from Fig. 2a that there is a departure from Minster and Jordan's model or any other rigid plate model when observed opening rates are averaged over the relatively short Brunhes time interval. The profile that yields the largest departure, at $29^{\circ}36'N$, corresponds to a misfit of 3.7 km in the position of the Brunhes reversal. This cannot be accounted for by the uncertainty in the model. To test the influence of the time scale which we have used we recomputed the opening rates (Fig. 2b) with the time scale of Mankinen and Dalrymple¹⁸. Although the discrepancy between observed and model rates is reduced, the data are clearly not compatible with rigid plate behaviour. In particular the opening rate observed at $29^{\circ}36'N$ for the Brunhes epoch is greater than that observed at $21^{\circ}06'N$ for the same time interval irrespective of the time scales. There is also evidence, in the region around $35^{\circ}N$ where several data points are closely spaced, for strong departures from rigid plate behaviour even for a 2.6 Myr averaging interval. We will not emphasize this result, however, in the absence of a detailed survey, because the differences in opening rates are of the same order as the uncertainties in the determination of rates. The fact that, when averaging is done over about 2.6 Myr, the observed rates fall in the slower portion of Minster and Jordan's band may be evidence that the angular velocity for the North America–Africa motion computed by Minster and Jordan is too high.

We note in Fig. 2 the differences in opening rate north and south of $30^{\circ}N$. One may be tempted to locate a plate boundary at this latitude but it finds no support in the geology. We also note in Fig. 2 that the opening rates during the Brunhes epoch are systematically greater than the rates averaged over longer time intervals. To examine in more detail the opening rate variations as a function of time, we present in Fig. 3 the spreading rates for the east and west limbs of the ridge for the Brunhes epoch (0–0.69 Myr BP), the latter part of the Matuyama epoch (0.69–1.78 Myr BP) and the period before the Gilsa event (before 1.87 Myr). To compute a spreading rate for the Brunhes epoch we assume that the location of maximum depth of the rift valley within the inner floor¹⁵ is the locus of accretion coinciding with zero age. Bathymetric information from Seabeam is very convenient for this purpose. The spreading rates for the other two time periods are derived directly from the models in Fig. 1. Two observations are apparent. First the distribution of spreading rates is approximately symmetric

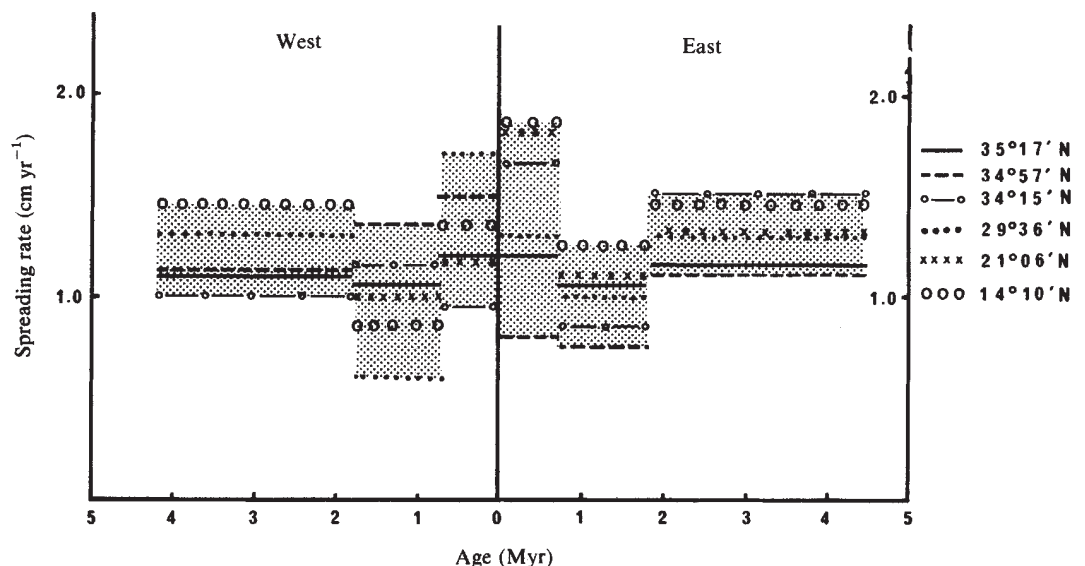
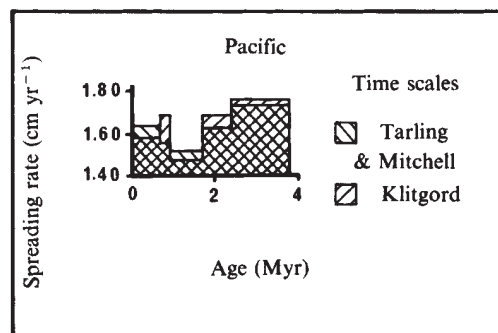
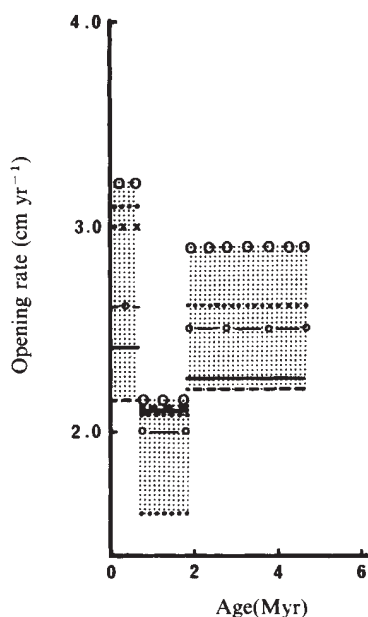


Fig. 3 Variation of spreading rates as a function of time. The spreading rates are keyed to their respective profiles. They are calculated for three time intervals: 0–0.69 Myr (Brunhes, anomaly 1), 0.69–1.78 Myr (Brunhes–Matuyama boundary to middle of anomaly 2), before 1.78 Myr.

Fig. 4 Variation of opening rates as a function of time (derived from Fig. 3). Inset: variation of opening rates along the East Pacific Rise and Juan de Fuca Ridge (after Rea and Scheidegger¹⁹).



about the ridge axis—no one side is accreting crust systematically faster than the other side. Second, the Brunhes epoch is characterized by a distinctly faster rate of spreading compared with time intervals up to 4.6 Myr. Similarly the latter part of the Matuyama epoch from 0.69 to 1.78 Myr BP is characterized by smaller spreading rates.

One can ignore the distinction between east and west accretion because of the approximate symmetry about zero age in Fig. 3 and examine the variation of total opening rates as a function of crustal age (Fig. 4). There is a definite decrease in accretion rates for the 0.69–1.78 Myr BP period. The decrease is correlated with a change in spreading direction (H. Schouten, personal communication) particularly near the Kane fracture zone at 24° N. Rea and Scheidegger¹⁹ also observed a definite slowing-down in plate accretion at the axis of the Juan de Fuca ridge (Fig. 4) which they linked to intraplate volcanism (Hawaii) and the dynamics of subduction¹⁹. We bring here some evidence that the spreading rate variations may be of global significance.

When accretion of the Mid-Atlantic Ridge is examined over a short time interval (<2 Myr) there is evidence that the oceanic plates do not behave rigidly. If we average the opening over a longer time interval (~2.6 Myr) the pattern of opening along the accreting plate boundary fits with that predicted by rigid plate behaviour. The evidence that we presented is derived from a rather simple modelling of magnetic anomalies but it is corroborated by more advanced modelling techniques¹³. The results are very sensitive to the position of the magnetic reversals but are not dependent on the number of sample points used in the 14–35° N interval. Thus, although we have used only a very limited data set, the departure from a rigid plate model is considered significant if one accepts the model results of Fig. 1. The modelling is strengthened by the fact that we have used magnetic profiles on two-dimensional bathymetry. The non-rigid opening between plates over short time intervals is not surprising and is consistent with the episodicity at the axis of mid-ocean ridges required by volcanic and tectonic data^{20–22}, geochronology^{23,24}, geochemistry and petrology (C. J. Allegre, personal communication) and hydrothermal activity (ref. 25 and R. D. Ballard, personal communication). It is clear that different segments of the Mid-Atlantic Ridge

display an independent behaviour and, with respect to relatively short time intervals, may show departure from characteristics typically associated with slow accretion. This may provide a mechanism for initiating small transform faults. The size of each independent segment is not known, however, nor is known the behaviour of the ridge accretionary process over shorter time intervals for want of additional magnetic markers in the Brunhes epoch. One will probably need high resolution bio-stratigraphy for this purpose.

There is also evidence that the fluctuations of spreading rate with time may be global in nature although a close look is needed at other parts of the Atlantic and Pacific oceans and also in the Indian Ocean.

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Ultrathin mycelia-forming organisms from submarine volcanic areas having an optimum growth temperature of 105 °C

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The most extremely thermophilic organisms known to date have been isolated from continental volcanic areas¹⁻³, and grow optimally between 70 and 85 °C. In the hope of finding organisms living at temperatures above 100 °C I have taken samples from the hot sea floor of a submarine solfataric field where, as a result of the high pressures liquid water is found that is hotter than 100 °C. Here I report that, from these samples, I isolated unusual disk-shaped prokaryotic organisms, connected by a network of thin hyphae, which grew at 100 °C in the presence of sulphur, hydrogen and carbon dioxide. The organisms could be successfully transferred to synthetic media and were strict anaerobes, growing optimally at 105 °C and not at all at 80 °C or below. However, they did survive for long periods at 4 °C and -20 °C in the absence of oxygen. During growth, H₂S was formed by sulphur reduction. Due to their extreme oxygen sensitivity, their unusual, irregular shape and primitive volcano-adapted metabolism, the novel organisms may represent a very ancient form of life existing in submarine volcanic areas in a water-supplied oxygen-free habitat, which has not changed for billions of years and which prevents competition with normal life due to prohibitively hot temperatures.

Extremely thermophilic organisms from submarine volcanic habitats have not been isolated previously. It has been assumed⁴ that due to their high salinity, hot marine waters may contain less caldactive organisms than the hot fresh waters occurring on the continent. Samples were collected from a submarine solfataric field close to the bay of Porto Levante, Vulcano Island, Italy. The sea floor was situated at a depth between 2 and 10 m and consisted mainly of sandy sediments with many small craters, and rock formations with gas-spewing, sulphur-encrusted holes and cracks. Temperatures of up to 103 °C were measured in the sediments and in the cracks and holes. Rubber gloves were used during sample collection to provide protection from the heat. The sediment was stirred as heavily and deeply as possible and material was then drawn into a syringe fitted with an enlarged inlet. The samples, still hot, were taken to the surface and transferred to storage bottles, which were immediately sealed with rubber stoppers, and then traces of oxygen were removed by injecting sodium dithionite⁵. Microscopic inspection of the samples revealed a small number of unusually flat organisms.

In the laboratory, sterile seawater was inoculated with the samples (5% inoculation) and incubated at 100 °C in the presence of sulphur and an H₂/CO₂ atmosphere (80:20 v/v; 2 bar pressure) with strict exclusion of oxygen⁶. Of the 21 samples taken from the sandy sediments and cracks, four yielded disk-shaped organisms which grew within 2 days. The original temperatures of the samples were all ~100 °C. The enrichment cultures were transferred successfully to a medium consisting of synthetic seawater supplemented with trace minerals⁶, KH₂PO₄ (0.5 g l⁻¹), and sulphur (15 g l⁻¹). Growth occurred only in the presence of H₂ and CO₂. H₂S, which was formed as an end product, was identified as cadmium sulphide. During growth, the sulphur flowers became greyish and appeared corroded. After 1 week, the sulphur supply was depleted. Uninoculated controls neither showed this phenomenon nor produced H₂S. These observations indicate that the novel organisms acquire their energy by a sulphur-hydrogen autotrophy similar

to that recently described for a rod-shaped bacterium from Iceland (ref. 7 and F. Fischer, W. Zillig and K. O. Stetter, in preparation).

The enrichment cultures were purified by serial dilution. During exponential growth (Fig. 1a,b), the pure cultures exhibited a doubling time of 550 min at 85 °C and of only 220 min at 100 °C (Fig. 1b). Surprisingly, the isolates grew optimally at 105 °C with a doubling time of 110 min (Fig. 1b). The isolates were transferred (1% inoculation) to fresh medium five times in succession and incubated at 105 °C. Microscopic inspection showed about the same high cell density in the last tube as in the first, indicating that the organisms multiplied at this temperature. At 110 °C, very weak growth occurred (not shown). It remains unclear whether this represents the upper temperature boundary, or whether, more probably, the reduction of growth was due to the sintering of the sulphur forming a lump and therefore rendering it inaccessible to the organisms. Below 80 °C, the isolates were unable to grow (not shown). However, they could be re-isolated from the original anaerobic samples, stored at 4 °C and at -20 °C over a period of at least 10 months. In the presence of oxygen, however, they were inactivated within hours.

Under the light microscope, the organisms appeared as flat disks, highly variable in diameter (from 0.3 to 2.5 µm), some having an indented, dish-like appearance. They were usually arranged in single file or in other formations with constant distances between each other. Under the electron microscope, most of the disks appeared to be connected by ultrathin rods up to 40 µm long (which are possibly hyphae; Fig. 2a) and only

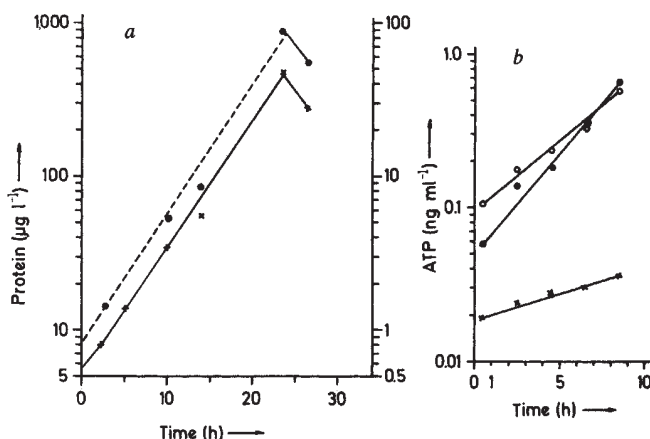


Fig. 1 Growth curves of the isolate PL-19. *a*, Growth in a 10-l fermenter at 95 °C: (x) ATP concentration; (•) protein concentration. The fermenter contained artificial seawater supplemented with 100 ml mineral mix⁷, 5 g KH₂PO₄, 150 g flowers of sulphur, 10 mg resazurin and 5 g Na₂S, pH 5.5 (H₂SO₄). It was gassed with a mixture of H₂/CO₂ (80:20 v/v; 6 l h⁻¹). Stirring was at 50 r.p.m. For protein determination, 850-ml portions were centrifuged (8,000 r.p.m., 30 min; WKF G 50 K centrifuge). The pellets were solubilized in 200 µl SDS (2% w/v) and boiled for 5 min. From this solution, protein was determined as described elsewhere⁹. For ATP determination, 1 ml of culture was centrifuged at 21,000 r.p.m. for 2 min in a J 21 Beckman centrifuge. The pellet was solubilized in 30 µl NRB solution (Lumac); 10 µl of this solution were mixed with 100 µl Lumit buffer (Lumac), 90 µl twice-distilled H₂O and finally with 100 µl enzyme (Lumit PM; Lumac). Then, the sample was immediately analysed with a scintillation counter (Biocounter M 2010; Lumac). As a reference, 1 ng ATP yielded 10,958 counts in the same conditions. For medium without inoculation, the background was only ~20 counts. The doubling time, determined by protein and ATP amplification, was 220 min. *b*, Growth in closed 25-ml serum tubes at various temperatures: (x) 85 °C; (o) 100 °C; (•) 105 °C. The tubes contained 10 ml culture medium as described above and were pressurized (2 bars) with H₂/CO₂ (80:20 v/v). The tubes were each inoculated with 0.5 ml of an overnight culture grown at the same temperature. Samples (1 ml) were withdrawn using a syringe and ATP determined as described above. The doubling times were 550, 220 and 110 min at 85 °C, 100 °C and 105 °C, respectively.

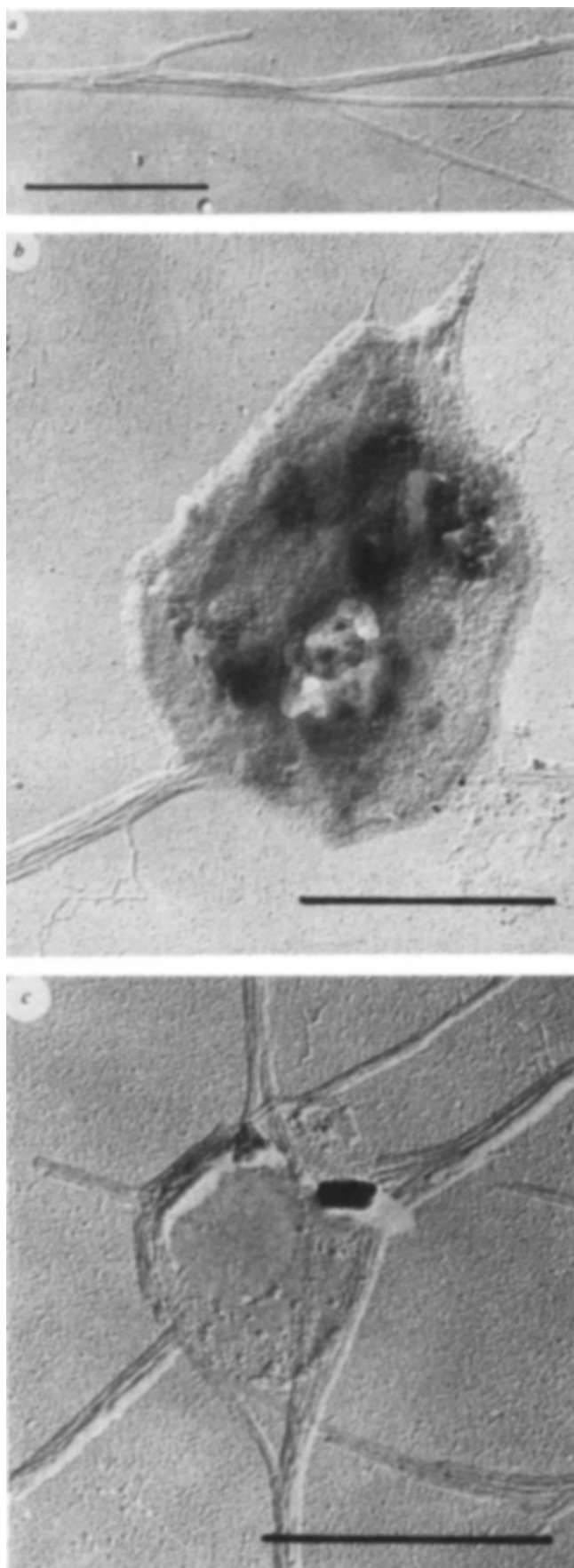


Fig. 2 Electron micrograph of isolate PL-19, platinum-shadowed. Scale bars, 1 μm . *a*, Bundle of hyphae; *b*, large disk-shaped cell situated laterally on a hypha; *c*, disk-shaped cell surrounded by hyphae.

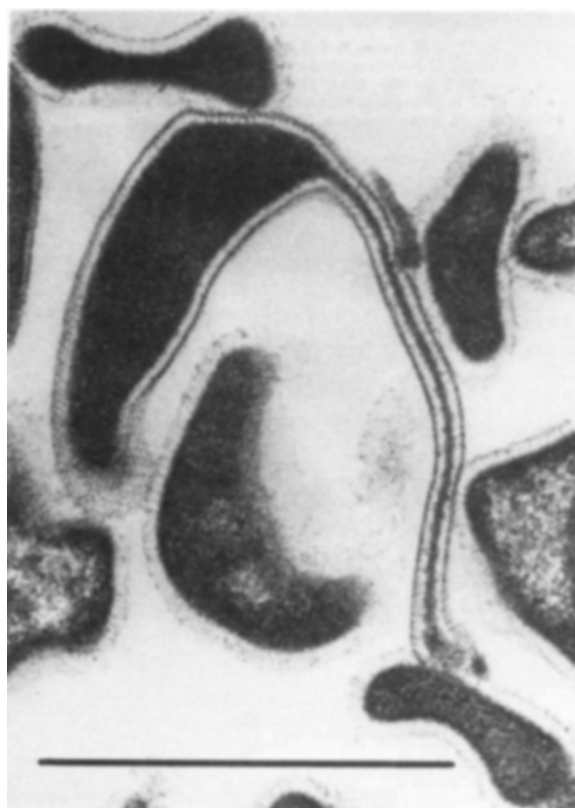


Fig. 3 Electron micrograph of thin sections of isolate PL-19. The sections were doubly contrasted with lead citrate and uranyl acetate. In the centre, a longitudinal section through a hypha attached to a disk-shaped cell is visible. Scale bar, 1 μm .

0.05–0.06 μm in diameter, thus they were not visible under the light microscope. The hyphae were often associated in bundles (Fig. 2*a*) or dispersed in loose networks covering areas of up to 10,000 μm^2 . The disks were situated laterally (Fig. 2*b*) and terminally on the hyphae. Sometimes, disks appeared to be embraced by several hyphae (Fig. 2*c*). Thin sections revealed that both the disks and the hyphae were surrounded by a membrane and a protein envelope consisting of subunits ~ 30 nm in diameter (Fig. 3). The hyphae terminated within the disks without septa (Fig. 3); they formed only when the culture was grown without movement. Gentle stirring (200 r.p.m.) led to the formation of disks having no hyphae, and vigorous stirring (800 r.p.m.) led to rapid inactivation of the culture. Both the hyphae and the disks contained thin pili-like appendices (Fig. 2*a,b*) up to 5 μm long and 10 nm in diameter; occasionally these were branched, often cross-linking two individuals.

The taxonomic position of the novel organism described here is unclear. Preliminary studies of the membrane lipids (T. Langworthy, personal communication) and the existence of an ADP-ribosylable protein in the crude extract (F. Klink, personal communication) suggest that it may be a novel archaeobacterium. As it is able to survive for long periods at low temperatures and due to its dependence on volcanic exhalations, this organism may be widely distributed in submarine volcanic areas.

After completion of this manuscript, Baross *et al.*⁸ described the existence of bacterial communities in hot deep sea waters, which can grow at 100 $^{\circ}\text{C}$ in the laboratory and produce H_2 , CH_4 , CO and N_2O . The media used, however, contained no sulphur and neither H_2S formation nor growth at 105 $^{\circ}\text{C}$ was observed. Therefore, these organisms are very different from those reported here.

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Function recovery following neural transplantation of embryonic septal nuclei in adult rats with septohippocampal lesions

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The remarkable capacity of transplanted embryonic neurones to innervate the hippocampal formation of mature recipients has been well documented, with the pattern of innervation being shown to be anatomically specific and to resemble normal connectivity^{1–3}. Although transplants are known to have functional consequences in other systems^{4–9}, information has yet to be obtained regarding the functional nature of embryonic septal transplants and the behavioural consequences of transplant innervation of the host hippocampal formation. We provide here evidence that a reinnervation of the hippocampal formation from cholinergic-rich septal transplants is functional in terms of the physiology of neural connectivity and that the newly formed connections can interact with an intrinsic afferent system, the perforant path. Moreover, we demonstrate that the reinnervation can aid in the partial recovery of the performance of a radial maze task that is thought to depend on the integrity of septohippocampal connections¹⁰. The behavioural performance of animals with transplants improved significantly compared with those without transplants when both groups were systemically injected with the acetylcholinesterase (AChE) inhibitor, physostigmine. These results suggest that neural transplants from embryonic tissue that reinnervate the hippocampal formation can form functional synaptic connections that can lead to the partial restoration of maze performance.

Each of three groups of female Sprague-Dawley rats (~180 g) received either bilateral lesions of the fimbria-fornix transecting all septohippocampal and commissural fibres followed by bilateral septal transplants (transplant group, $n = 8$), or similar bilateral lesions of the fimbria-fornix without transplants (lesion group, $n = 7$), or sham operations (control group, $n = 7$). The transplanted septal-diagonal band area was taken from 16–17-day-old embryos (crown-rump length 15–20 mm) of the same strain and implanted into the cavity created by the suction lesion of the fimbria-fornix. The cavity exposed a vascular bed overlying the anterior thalamus, upon which the embryonic tissue was placed as previously described¹¹. To permit the transplant to reinnervate the hippocampal formation, the animals were allowed to survive for 7 months before behavioural testing.

The rats were tested with a raised eight-arm radial maze, as Olton *et al.*¹⁰ have shown that the performance of this task is disrupted by damage to septohippocampal afferents. Rats were stabilized on a 23-h food deprivation schedule, which was maintained throughout behavioural testing. During each daily trial, each arm of the maze was baited and the rat was placed in the central arena, free to enter any arm in any order. Once the animal had entered an arm and consumed the pellet it received no food on subsequent visits to that arm. Optimal performance by the rat involved choosing only arms that had not previously been visited. The performance of each animal was determined by the number of correct arms (those arms that it had not previously visited during the trial) selected out of the first eight choices.

Analysis of maze performance during the first 30 days of testing revealed that the three groups differed significantly ($F = 4.93$ with 2, 19 d.f.). The control animals showed a gradual improvement over the first 15 days of testing and stabilized thereafter (linear trend over 30 days, $F = 12.64$ with 1, 54 d.f., $P < 0.001$). The performance of animals with bilateral lesions did not improve with repeated trials (analysis of linear trend not significant, $F = 0.02$ with 1, 54 d.f.). The animals with septal transplants, however, did improve over the 30 days of testing (linear trend $F = 4.26$ with 1, 63 d.f., $P < 0.05$), but their overall improvement was not as great as that of the control group and their overall performance did not differ significantly from the bilaterally lesioned animals.

We postulated that the lack of overall improvement in the transplant group when compared with the lesion group might be due to insufficient cholinergic innervation. To test this hypothesis, all animals were tested for an additional 8 days during which they were alternately injected (intraperitoneally, i.p.) with 0.05 mg per kg of physostigmine, an acetylcholinesterase inhibitor, or with an equal volume of saline. Physostigmine has previously been shown to enhance discrimination performance^{12,13}.

Figure 1 shows the maze performance of the three groups under saline and physostigmine; the interaction between groups and drug was significant ($F = 4.13$ with 2, 18 d.f., $P < 0.05$). Both the control group and animals with transplants showed enhanced maze performance under the drug (planned orthogonal comparisons, $P < 0.005$ and $P < 0.001$, respectively) whereas the bilaterally lesioned group showed no improvement ($P > 0.25$). These results indicate that embryonic septal transplants can aid in the partial restoration of maze performance.

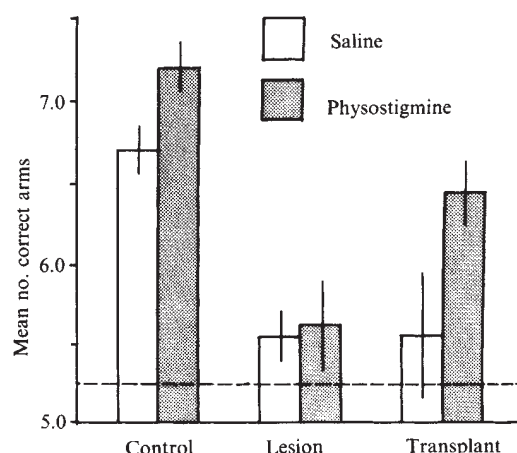


Fig. 1 Mean maze performance of rats with control sham operations ($n = 7$), fimbria-fornix lesions ($n = 7$) and septal transplants ($n = 8$) following injections of isotonic saline or 0.05 mg per kg physostigmine; vertical bars represent s.e.m. Both the control and transplant groups, but not the lesion group, showed significantly improved performance following injection of the cholinesterase inhibitor ($P < 0.05$, $P < 0.001$, and $P > 0.25$, respectively). The horizontal dashed line in each graph represents the random performance level.

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In particular, the enhanced maze performance with physostigmine administration in the transplant and control group, but not the lesion group, indicates that recovery is dependent on the re-formed cholinergic connections.

In addition to the extent of the cholinergic reinnervation, the complexity of the eight-arm maze task might affect the performance of the transplant and lesion groups. Roberts and Dale¹⁴ have shown that rats tested with such a radial maze begin to adopt strategies when the memory requirements are quite demanding. Although animals of both groups tended to adopt strategies, the strategy generally adopted was to select alternate arms. This proved to be an unsuccessful strategy in that after the first four correct choices, rats would then enter arms that had been visited previously. Consequently, if an animal persisted in this strategy, it would err in its next four choices. Interestingly, animals in our control group did not have a tendency to adopt strategies, even successful strategies such as selecting adjacent arms as reported by Roberts and Dale¹⁴. When tested in maze tasks with less complexity, animals with transplants have been shown to perform significantly better than bilaterally lesioned animals without the aid of physostigmine¹⁵.

On completion of the behavioural trials, five animals with septal transplants and one animal with fimbria-fornix lesion alone were used for electrophysiological analysis. The region of the dentate gyrus within the hippocampal formation was analysed because of the dense reinnervation in this area by the transplant as revealed by preliminary histological studies using AChE histochemistry. Furthermore, perforant path fibres from the entorhinal cortex also project to the granule cells of the dentate, and when electrically stimulated, evoke characteristic field potentials in the dendritic and somatic zones of the granule cell layer^{16,17} (Fig. 2a, left panel).

The rats were anaesthetized with urethane (1.5 g per kg, i.p.) and placed in a stereotaxic apparatus where the transplant and surrounding neocortex were exposed and kept under saline. A field potential recording electrode was placed within the granule cell region of the dentate gyrus (anterior -3.5 mm, lateral 2.5 mm, vertical -2.7 to -3.7 mm with respect to bregma, incisor bar at -4.0 mm), and stimulating electrodes were placed on the transplant and in the angular bundle of the perforant path (anterior -8.1 mm, lateral -2.0 mm, vertical -2.0 to -3.0 mm). In all five animals studied with septal transplants, stimulation of the transplant resulted in positive-going monophasic field potential responses in the granule cell layer of the dentate (Fig. 2a, right panel, depth at 0 μ m). The stimulation of the tissue surrounding the cavity in the lesioned animal without a transplant did not evoke field potential responses in the dentate.

Depth profile analysis of field potentials provided further evidence for the functional nature of the transplant innervation of the host. The depth profile (Fig. 2a) shows the field potentials evoked by alternating perforant path (left panel) and transplant stimulation (right panel) at successive depths during a single electrode penetration through the laminae of the dentate gyrus. The perforant path-evoked synaptic wave in the dentate exhibited a sharp polarity reversal as the electrode was moved from the dendritic zone down into the cell body layer. This characteristic reversal of the perforant path-evoked synaptic field potential in the dentate reflects the pattern of monosynaptic innervation from the perforant path which is confined to the mid and outer regions of the granule cell dendritic layer^{18,19}. The transplant-evoked response during the same traverse continued to exhibit positive-going field potentials. Superimposed on these potentials were negative deflections which were observed when the recording electrode was situated in the dendritic region.

The innervation arising from the transplant was examined further to test its ability to interact with the fibre innervation arising from the perforant path. In this set of experiments, we set the stimulus intensity for the activation of perforant path fibres at a level that was insufficient to evoke the synchronous

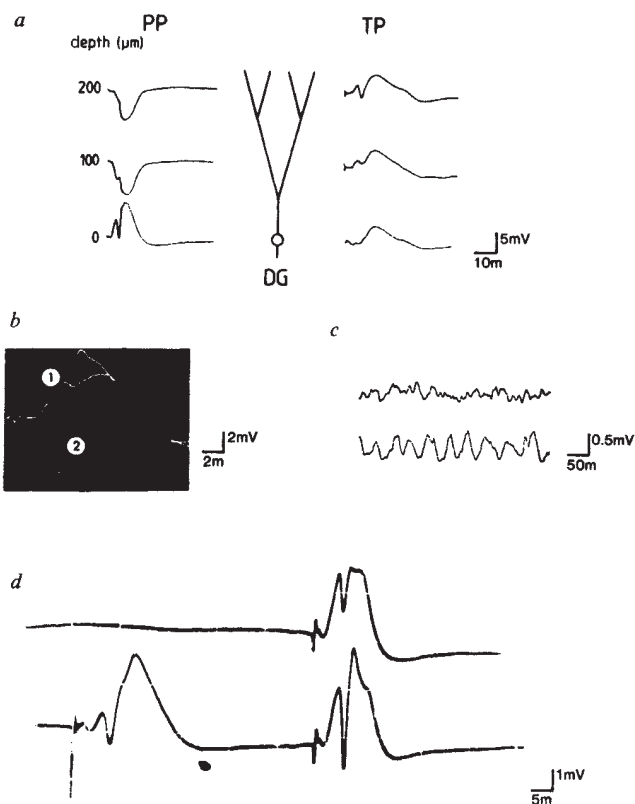


Fig. 2 Electrophysiology of dentate innervation. *a*, Vertical field potential profile of perforant path (PP) and transplant (TP) evoked responses through dentate laminae. Recording electrode positions relative to granule cell body layer (DG) are indicated. *b*, Sub-threshold PP-dentate-evoked response (trace 1) and potentiated PP-dentate-evoked response (trace 2) potentiated by TP stimulation preceding PP stimulation by 50 ms. *c*, Monopolar recording of slow-wave activity (filters 0.1–100 Hz) from theta-generating zone of the dentate molecular layer 15 min after injection of physostigmine (0.05 mg per kg). Slow-wave activity in the theta-frequency range (6–10 Hz) is absent. An irregular pattern predominates (upper trace), interspersed with periods of regular sinusoidal activity at higher frequencies (15–20 Hz, lower trace). *d*, Field potentials from animal with intact septohippocampal connections. Trace 1: submaximal PP-dentate-evoked response. Trace 2: septo-dentate-evoked response (left) followed by potentiated PP-dentate-evoked response (right). All traces shown are positive up in polarity and calibrated individually.

discharge of granule cells or population spike (Fig. 2b, trace 1). When the stimulation of the perforant path was preceded by the stimulation of the transplant, a prominent perforant path evoked population spike was observed (Fig. 2b, trace 2) which was maximal with inter-stimulus intervals in the range of 30–70 ms.

Similar evoked responses and heterosynaptic interactions were observed in control animals with intact septohippocampal connections. Stimulating electrodes were placed in the dorsal aspect of the medial septum and the angular bundle of the perforant path in rats anaesthetized with urethane (1.5 g per kg, i.p.). A recording electrode was placed in the granule cell layer of the dentate. Electrical stimulation of the perforant path alone evoked a population spike response as shown in trace 1 of Fig. 2d. When the stimulation of the perforant path was preceded by the stimulation of the dorsal aspect of the medial septum (50 ms interstimulus interval) the perforant path-dentate-evoked response was markedly potentiated (Fig. 2d, trace 2). The first response shown on trace 2 of Fig. 2 is the positive-going dentate field potential resulting from the stimulation of the medial septum. Superimposed on this is a negative deflection probably due to the synchronous activation

of granule cells. The second response of trace 2 is the potentiated perforant path-dentate population spike. Septo-dentate-evoked responses have been demonstrated by other investigators²⁰, as well as heterosynaptic interactions within the hippocampal formation^{21,22}. Note that in contrast to the presence of the negative-going septo-dentate spike as recorded in the granule cell layer of control animals, animals with septal transplants did not exhibit such spikes when the transplant was stimulated. The absence of the negative spike in these animals may be due to an insufficient convergence of fibres from the transplant required to synchronously activate the granule cells.

In the final set of electrophysiological experiments with the transplant group, we attempted to induce low-frequency (6–10 Hz) rhythmical electrical activity in the hippocampal formation (theta waves), which is thought to be paced by the medial septum^{23,24}. Injections of physostigmine (0.05 mg per kg, i.p.) were capable of inducing rhythmical activity, but at frequencies higher than theta activity (Fig. 2c). The lack of theta activity may reflect an insufficient cholinergic reinnervation and/or the absence of other non-cholinergic afferents which were eliminated during the lesioning procedure. This, in turn, may account for the incomplete restoration of maze behaviour.

After the behavioural and electrophysiological studies animals were processed for AChE histochemistry. The animals with septal transplants, but not those with lesions alone, exhibited AChE-positive staining in the dorsal hippocampus with a laminar distribution typical of the cholinergic innervation in intact animals, as has been described in detail elsewhere³.

Thus, the present study provides evidence that septal transplants are capable of forming physiologically viable connections with the hippocampal formation of the recipient. These connections, in turn, can enhance the efficacy of the perforant path input to the dentate gyrus. The cholinergic reinnervation, moreover, seems to account for the partial recovery of performance as examined with an eight-arm radial maze. The behavioural manifestations of the transplants, however, must also be viewed in the light of investigations demonstrating the ability of intracerebral transplants to become innervated by the host tissue^{25–27}. Heterosynaptic interactions and interconnections indicate that the host and transplant are capable of integration in a physiological and anatomical sense. Such an integration provides a framework for the processing of neural information mediating the partial recovery of behavioural responses.

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Effector T lymphocyte line cells migrate to the thymus and persist there

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The thymus gland has been known for some time to be the central organ of differentiation of T lymphocytes^{1,2}. Stem cells migrate into the thymus from the bone marrow, differentiate and, as competent T lymphocytes, disperse from the thymus to the periphery, where contact with specific antigen induces immune reactivity². The traffic of T lymphocytes between the thymus and periphery has been thought to be unidirectional and, unless the cells are leukaemic³ or the thymus gland has been irradiated⁴, re-entry of peripheral T lymphocytes has not been detected³. We now report that cells from lines of functionally active T lymphocytes reactive to self or foreign antigens can migrate back into the normal thymus gland and persist there for relatively long periods in a quiescent state until activated by contact with antigen. Hence, in addition to being the seat of T lymphocyte differentiation, the thymus is open to two-way traffic with the periphery and may function as a repository of immunological memory.

The experiments described here were prompted by our investigation of the behaviour of cells of functional T lymphocyte lines. We have found that lines of rat T lymphocytes specific for the basic protein of myelin could cause experimental autoimmune encephalomyelitis (EAE) upon intravenous inoculation into naive recipient rats^{5,6}. Moreover, cells from such lines, attenuated by treatment with mitomycin C or irradiation, could be used for vaccinating rats against the induction of active EAE by subsequent immunization of the rats to myelin basic protein^{7,8}. To study the migrations of such cells, we labelled them with radioactive chromium-51, injected them into rats intravenously and measured the accumulation of radioactivity as an indication of the accumulation of the injected cells in various organs. We found that anti-myelin basic protein cells accumulated in the brain and spinal cord about one day before the onset of EAE (Fig. 1). Cells from lines reactive to other antigens, such as tuberculin (PPD), did not accumulate in the central nervous system. However, Fig. 1 shows that about 1% of injected anti-myelin basic protein or anti-PPD line cells accumulated in the thymus glands of recipient rats beginning 3 days after intravenous inoculation. Accumulation in the thymus was detectable only if the cells were intact and had been activated by incubation with specific antigen before inoculation. Non-activated cells did not enter the thymus, nor did activated and irradiated cells. It is noteworthy that nonactivated, or activated and irradiated cells also did not mediate EAE⁷ or transfer delayed hypersensitivity (manuscript in preparation). Thus, migration to the thymus appeared to be correlated with the state of the T lymphocytes and their capacity to mediate specific immunological effects.

Experiments were then designed to answer three questions: (1) do the returned T lymphocytes persist in the thymus, (2) are they capable of responding to specific antigen and (3) do they include potential effector cells? We injected female anti-myelin basic protein or anti-PPD line cells into syngeneic male recipient rats, and two months later we assayed the proliferative responses of their thymus cells to myelin basic protein or PPD.

The thymus glands used in the experiments were dissected free of the lymph nodes imbedded in their postero-lateral portions. The results are shown in Table 1. Control thymus cells from

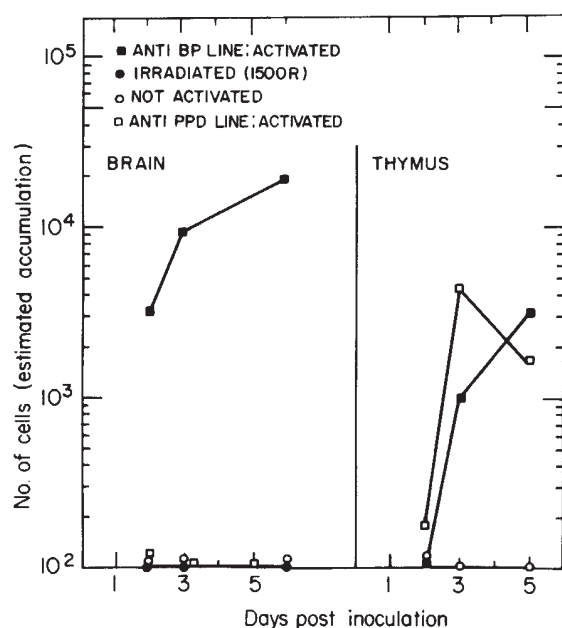


Fig. 1 Activated anti-myelin basic protein cells accumulate in the brain and thymus; activated anti-PPD line cells accumulate in the thymus. Lines of T lymphocytes specifically responsive to myelin basic protein or to PPD were raised from lymph node cells of 3-month old female Fischer rats that had been immunized with myelin basic protein-CFA^{5,6}. The cells were maintained in medium containing T cell growth factor⁹ and some of the cells were activated by incubation with their respective antigen, myelin basic protein or PPD in the presence of irradiated (1,500 rad) normal thymus accessory cells for 72 h (refs. 5, 6): some of these activated cells were irradiated with 1,500 rad. The line cells were labelled¹⁰ by incubating 10^7 cells per ml Eagle's medium containing ^{51}Cr as sodium chromate (Amersham). The cells were washed by centrifugation in medium. Groups of 6 male Fischer rats were then inoculated into the tail vein with 3×10^6 labelled cells from lines of each type. On days 2, 3, 5 or 6, two rats of each group were killed and the accumulated line cells in the brains and thymuses were estimated by the c.p.m. emanating from each whole organ measured in a gamma counter. Differences between the organs of the two rats assayed at each time point were negligible. The number of cells was computed as the c.p.m. divided by the c.p.m. per cell in the inoculum. Similar results were obtained in four consecutive experiments.

naive rats showed no response to myelin basic protein and only a weak response to PPD (SI = 2). The thymus cells of rats that had been inoculated intravenously with activated anti-myelin basic protein line cells and had recovered from EAE^{8,11} responded weakly to myelin basic protein (SI = 3). The slight response to PPD of these thymus cells was about the same as that of naive control rats (SI = 2). Injection of anti-myelin basic protein cells did not produce a response to PPD. In contrast, thymus cells from rats that had been inoculated with activated anti-PPD cells showed a response to PPD (SI = 6) but not to myelin basic protein. Nonactivated anti-PPD cells produced no response.

To determine the immunospecificity of the cells responding to myelin basic protein and to determine their origin, they were grown in medium containing T-cell growth factor (IL-2)⁹ with alternating periods of stimulation with myelin basic protein until they developed into a stable line of T lymphocytes⁶. Accessory cells added during stimulation with myelin basic protein were of male origin, as were the recipient rats from whom the thymuses were taken for culture. Thus, only the original line cells inoculated over 2 months earlier were of female origin.

Table 1 shows that cells recovered from anti-myelin basic protein lines responded strongly and specifically to the antigen *in vitro* (SI = 13), and mediated EAE upon intravenous inoculation into naive Fischer rats. Furthermore, analysis of the chromosomes of the recovered anti-myelin basic protein cells showed that they all had the female karyotype of the original line. Therefore, the antigen-specific cells detectable in the thymus 2 months after inoculation and spontaneous recovery from EAE were cells of the donor EAE effector line. Similar results were obtained in experiments using rats and line cells of the Lewis rat strain.

These findings indicate that competent, specifically sensitized T lymphocytes can re-enter the thymus and persist there. Thus, the thymus is connected to the periphery by a two-way flow of potential effector lymphocytes. The T lymphocytes that leave the thymus appear to be those induced to differentiate from precursors by contact with epithelial cells of the thymic stroma¹³ or with macrophage-like cells¹⁴. This stage of differentiation is not dependent on clonal recognition of specific antigens but may involve the acquisition of the restriction of antigen recognition to association with particular products of the major histocompatibility complex^{15,16}. In contrast, the T lymphocytes that return to the thymus from the periphery appear to be induced to do so in response to antigen. The cell lines used in these experiments were developed from cells of antigen-primed rats by their proliferative responses to specific antigens and by their ability to respond to T cell growth factor^{5,6}. Furthermore, the cells did not return to the thymus and persist there unless

Table 1 Activated female anti-myelin basic protein T lymphocyte line cells persist in male thymus and can be recovered

Female line cells inoculated into male rats		Thymus cells		Recovered anti-myelin basic protein cells				
Specificity	Treatment	Proliferation Δ c.p.m. (SI)		Proliferation Δ c.p.m. (SI)		Incidence of EAE	Karyotype	
		Myelin basic protein	PPD	Myelin basic protein	PPD		Male	Female
None	None	95 (1)	921 (2)					
Anti-myelin basic protein	Activated	3,156 (3)	1,376 (2)	82,051 (13)	0 (1)	5/5	0/20	20/20
	Irradiated	0 (1)	880 (1)					
	Nonactivated	0 (1)	995 (2)					
Anti-PPD	Activated	0 (1)	4,984 (6)					
	Nonactivated	0 (1)	950 (2)					

Proliferative responses to myelin basic protein or to PPD of thymus cells from groups of five male 3-month old Fischer rats were measured^{5,8}. Some of the groups had been inoculated i.v. 2 months earlier with 3×10^6 female anti-myelin basic protein or anti-PPD line cells^{5,6}. Cells from the lines were either nonactivated, activated or activated and irradiated (see Fig. 1 legend). Rats that had been inoculated with anti-myelin basic protein line cells developed EAE 4 days later and recovered spontaneously^{6,11}. An anti-myelin basic protein line was recovered from pooled thymuses free of lymphnodes of the male rats that had been inoculated with the female activated anti-myelin basic protein line cells⁶. The proliferative responses of these line cells to myelin basic protein and to PPD were tested⁶, as was their ability to mediate EAE in naive recipient rats. The karyotype of the anti-myelin basic protein line cells was studied in 20 cells¹².

they had had contact with their specific antigen just before inoculation. Thus, the capacity of anti-myelin basic protein T lymphocytes to return to the thymus, to persist there and to mediate EAE appeared to be part of the programme of differentiation induced by the antigen. In their persistence and response to specific recall, these cells are a token of immunological memory.

We also have been able to recover EAE effector T lymphocytes from the thymuses of rats that had recovered from EAE induced by active immunization to BP in adjuvant¹¹. Thus, the thymic immigration of effector T lymphocytes would seem to include cells generated endogenously as well as exogenous line cells. The anatomical site of residence in the thymus of the returned T lymphocytes is unknown, but it is probable that they comprise part of the pool of relatively mature longlived cells residing in the medulla¹⁷.

The present observation raises a number of questions. Are all populations of sensitized lymphocytes represented in the

thymus? Are some sensitized T lymphocytes specifically destined to return to the thymus because they bear particular receptors, or is the return a random process in which all sets of T lymphocytes participate equally? Does the persistence in the thymus of these returned cells have a role in immunological memory? Why and how are persistent autoimmune effector T lymphocytes kept quiescent? Is activation of such cells a factor in the progression or exacerbation of autoimmune diseases? Or, on the contrary, does the persistence of autoimmune cells help maintain self-tolerance by priming anti-idiotypic networks¹⁸?

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Human hybridomas secreting anti-islet autoantibodies

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Serum from patients with type I diabetes mellitus contains antibodies which react with all islet cells on sections of human pancreas¹, normal rat islet cell surface antigens², antigens expressed by islet cell tumours³, β -cell-specific surface antigens⁴ and A cells of sections of human pancreas⁵. Due in part to the low titre of anti-islet antibodies, the biochemistry of the relevant islet cell antigens is poorly understood⁶. Our laboratory has begun to develop and define a series of murine monoclonal anti-islet antibodies⁷⁻⁹. In addition, we have begun to explore the possibility of producing human hybridomas secreting monoclonal anti-islet antibodies by fusing lymphocytes from patients having type I diabetes mellitus with human myeloma cell lines. We describe here the isolation of human hybridomas that secrete human monoclonal anti-islet antibody. The availability of hybridoma clones secreting autoantibody should result in a better understanding of the mechanisms resulting in the expression of human autoantibodies.

After two unsuccessful fusions in which no anti-islet antibodies were detected, lymphocytes from 20 ml of blood from a patient with type I diabetes mellitus of 5 months' duration were fused using polyethylene glycol with the GM1500 6TG-2 myeloma-derived cell line (10^7 myeloma cells, $\sim 2 \times 10^7$ lymphocytes)¹⁰. Using frozen sectioned pancreas and fluorescein-

conjugated rabbit anti-IgG antibodies, the patient's serum reacted strongly with all islet cells in a typical anti-islet pattern, while with fluorescein-conjugated anti-IgM antibody it reacted selectively with peripheral islet cells, suggestive of an A-cell pattern⁵. After fusion, the resulting cells were distributed into 24 wells of a Linbro plate; colonies developed in 22 of the wells. Supernatants from these 22 hybrid cultures were tested for the presence of anti-islet antibodies reacting either with the cell surface of viable rat insulinoma cells or with islets of Bouin's fixed human pancreas. No supernatants contained an anti-cell-surface antibody.

Only one of the 22 hybrids, B6, produced an antibody which reacted with islets of Bouin's fixed pancreas. As shown in Fig. 1b, antibody B6, similarly to the anti-islet antibodies of patients with type I diabetes mellitus (Fig. 1a), reacts preferentially with islet cells of Bouin's fixed human type 0 pancreas. Preincubation of antibody B6 with insulin does not block its binding (Fig. 1c). Figure 1d is a fluorescein photomicrograph of a pancreas section incubated with antibody GM607, a negative control monoclonal IgM antibody (Fig. 2), as contrasted with the parental myeloma (IgG)¹⁰. We have subcloned the B6 hybrid cells by limiting dilution without feeder layers into microwells of 96-well Linbro

Table 1 HLA-antigen assignments

	A	B	C
GM 1500 myeloma	A2, Aw19 (?33)	B27, B18	Bw6
Patient	A2	B27, Bw16 (w39)	Bw4, Bw6
B6 cell line	A2, Aw19 (?33)	B27, B18,	Bw4, Bw6
clone 10-13		Bw16 (w39)	

plates. All six hybrid clones produced and secreted IgM ($\sim 0.4 \mu\text{g ml}^{-1}$ of antibodies in spent culture supernatant) that reacted with islet cells in the same way as the parental B6 hybrid (Fig. 3). As all hybrid clones derived from B6 expressed anti-islet IgM, it is likely that hybrid B6 was itself a clone. The fact that all B6 clones were producing anti-islet IgM to the same extent (Fig. 3) and that B6 hybrids and its clones have maintained the production of the same amounts of anti-islet IgM after approximately 1 yr in tissue culture strongly suggest that such human-human hybridomas are quite stable. To

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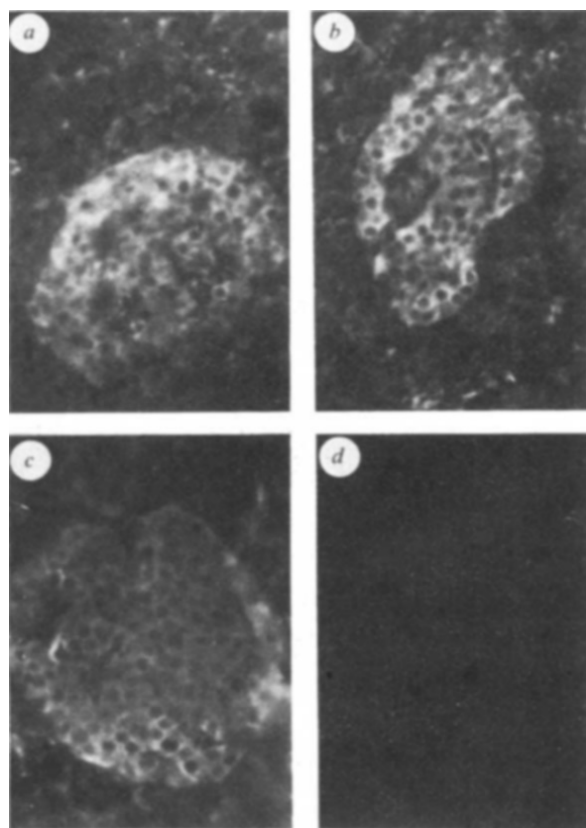


Fig. 1 Indirect immunofluorescence with Bouin's fixed human pancreas. Lymphocytes from 20 ml of heparinized blood from a child with type I diabetes were fused with the GM1500 6TG-2 human myeloma cell line using polyethylene glycol by methods previously described¹⁰. Supernatants from cultures were screened for antibodies reacting with the cell surface of a cloned rat insulinoma cell line using a ⁵¹Cr-release assay³, an ¹²⁵I-protein A radioassay¹³ and fluorescein-coupled anti-antibodies in an indirect immunofluorescence assay. In addition, supernatants were incubated with 4- μ m thick sections of Bouin's fixed human pancreas (type 0), and anti-islet antibodies were detected using fluorescein-coupled anti-human antibody (Miles). Supernatants from the GM607 (IgM) and the GM1500 (IgG) human myelomas were used as negative controls. Culture B6, which produces an anti-islet antibody, has been cloned by limiting dilution, and has stably produced antibody in culture for >1 yr. Antibody B6 is produced by cells grown in RPMI medium supplemented with 10% newborn calf serum, hypoxanthine (10^{-4} M) and thymidine (1.6×10^{-5} M). Antibody is collected from culture supernatants by adding an equal volume of saturated $(\text{NH}_4)_2\text{SO}_4$. The precipitate is resuspended in 1/20th its original volume of Dulbecco's phosphate-buffered saline (PBS) and then dialysed against PBS. Antibody B6 is then affinity-purified using rabbit anti-human antibody coupled to Sepharose. The antibody is eluted from this affinity column in 1-ml fractions with a glycine-HCl buffer (pH 2.28, 0.05 M glycine HCl, 0.15 M NaCl) neutralized with 20 μ l of 1 M Tris and dialysed against PBS. *a*, Serum from a patient with type I diabetes; *b*, antibody B6; *c*, antibody B6 after incubation with insulin; *d*, control negative antibody GM607. $\times 188$.

confirm that the cells producing antibody B6 are hybrid cells, the cell line B6 and the B6-derived positive clone 10-13, the patient's lymphocytes and the GM1500 parental cell line were HLA-typed by Dr Frances Ward (Duke University). As shown in Table 1, B6 and clone 10-13 cells contain the HLA antigens of both the myeloma-derived parent and the patient.

Anti-islet antibodies reacting with sectioned human pancreas are traditionally assayed using frozen sections of human pancreas¹ and only recently have Bouin's fixed sections been used to detect anti-islet antibodies¹¹. We therefore studied the reaction of antibody B6 with frozen sections of human pancreas, both with and without acetone fixation (Fig. 4). Antibody B6 on frozen sections reacted only with cells that also stained with antibody to glucagon (A cells), whereas with Bouin's fixed sections, all islet cells reacted. Similar to results using anti-insulin antibody, double immunofluorescence with anti-somatostatin and anti-human pancreatic polypeptide antibodies



Fig. 2 10% Polyacrylamide gel run in reducing conditions with 10% SDS and stained with Coomassie blue (R250). Left gel, affinity-purified antibody B6; right gel, molecular weight standards (200,000; 116,250; 92,500; 66,200; 45,000). Heavy chain of antibody B6 has the molecular weight of an IgM heavy chain.

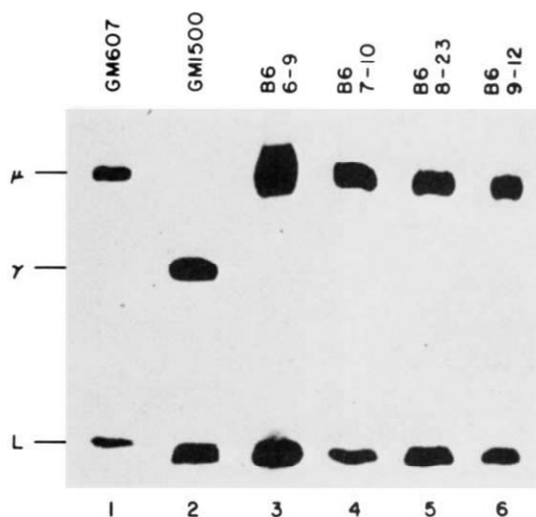
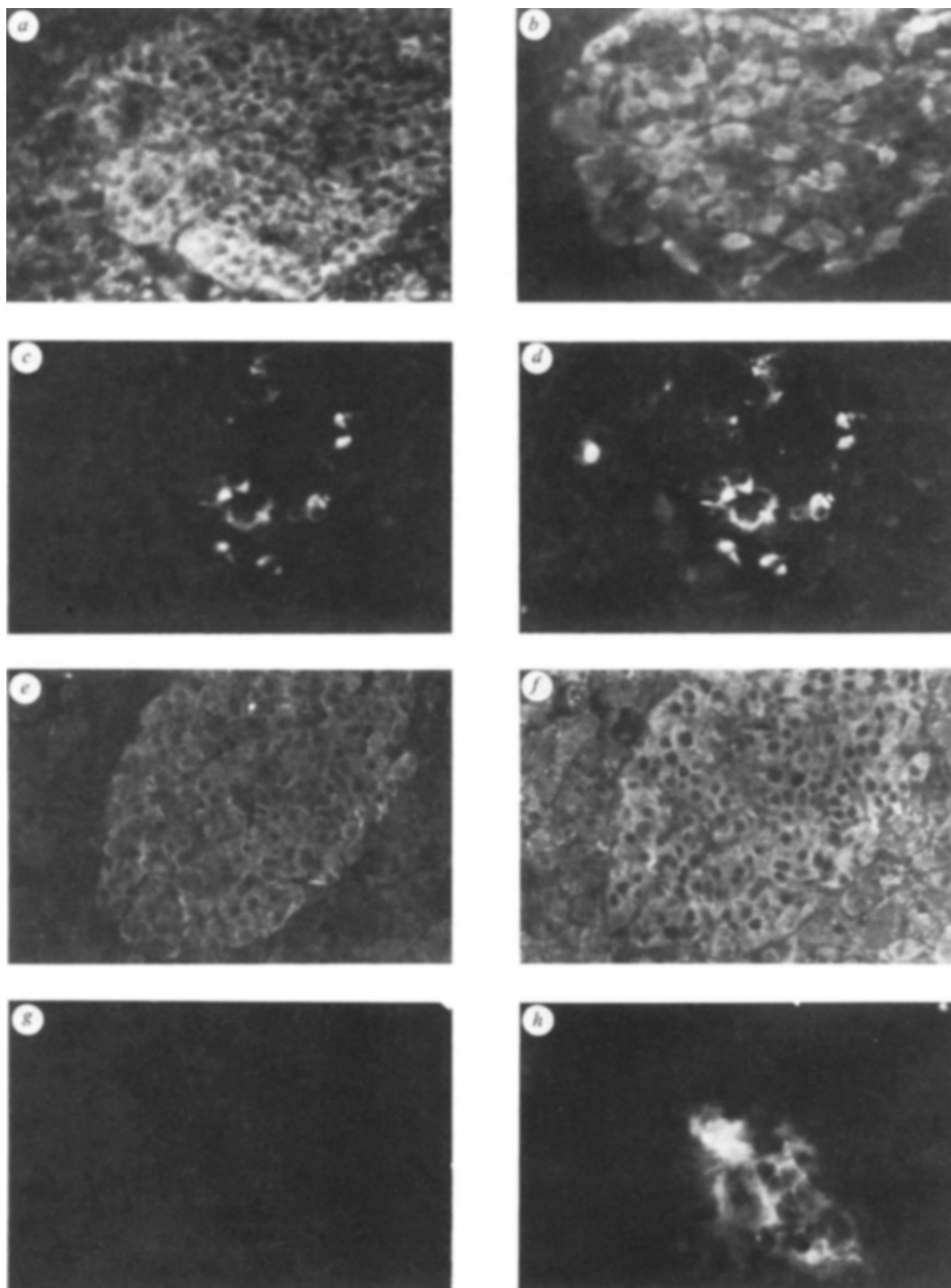


Fig. 3 Immunoprecipitation of immunoglobulin chains produced by B6 clones. The immunoprecipitation of the human immunoglobulin chains was carried out as described previously by using anti-human immunoglobulin rabbit antisera¹⁰. The human chains were separated by SDS-polyacrylamide gel electrophoresis¹⁰. Lane 1, human immunoglobulin chains secreted by GM607 human lymphoblastoid cells¹⁰; lane 2, human immunoglobulin chains secreted by the GM1500 human myeloma-derived parent; lanes 3-6, human immunoglobulin chains secreted by four clones of the B6 hybrid. Identical results were obtained with two additional clones and the parental B6 hybrid.

showed no overlap of the cells reacting with antibody B6 (not shown).

The technology for the production of murine and rat monoclonal antibodies is well developed, and such techniques have

Fig. 4 Double indirect immunofluorescence using Bouin's fixed (a, b, e, f) and frozen sections (c, d, g, h) of human type 0 pancreas. In addition to Bouin's fixed pancreas we studied the reaction of antibody B6 with acetone or unfixed 4- μ m frozen sections of human pancreas. Tissue sections were incubated with 25 μ l of antibody (B6 or control) for 30 min at room temperature, washed three times with PBS (5 min per wash), then incubated with a 1:50 dilution of fluorescein-coupled affinity-purified anti-human antibody for 30 min, washed three times with PBS and then viewed with a Leitz diavert microscope equipped for epifluorescence. For determination of the islet cell type reacting with antibody B6, fluorescently stained sections were incubated with the appropriate anti-hormonal antibody, washed and then incubated with rhodamine-coupled antibody. Rabbit antiglucagon antibody and rabbit anti-somatostatin antibody (provided by M. Appel, Worcester, Mass.), rabbit anti-human pancreatic polypeptide antibody (Lilly) and guinea pig anti-insulin antibody (Cappel) were used. After a 30-min incubation at room temperature with the anti-hormonal antibody diluted 1:50 in PBS, 1% albumin, the sections were washed with PBS and incubated for an additional 30 min with a 1:50 dilution of appropriate anti-rabbit or anti-guinea pig rhodamine-labelled antisera. By sequentially viewing the section with the 'rhodamine' and 'fluorescein' fluorescent filters of our Leitz microscope, the cell type reacting with antibody B6 was determined. Human monoclonal antibodies [GM607 (IgM) and GM1500 (IgG)], normal rabbit serum, or normal guinea pig serum served as negative controls. Sections were incubated with monoclonal antibody B6, washed, incubated with anti-glucagon antibody (a-d) or anti-insulin antibody (e-h), washed and then incubated with rhodamine-coupled anti-rabbit (a-d) or anti-guinea pig (e-h) antibody and then viewed with a Leitz microscope equipped for epifluorescence with either the fluorescein (a, c, e, g to detect B6 binding) or rhodamine filter (b, d, f, h to detect hormonal content). $\times 143$.



been widely applied¹². The production of human monoclonal antibodies by human-human 'hybridomas' has been more difficult. Ours is the first report of a human hybridoma producing a monoclonal autoantibody reacting with islet cells.

The pattern of reaction of antibody B6 with Bouin's fixed pancreas and frozen sections of pancreas is dramatically different. Bouin's fixation involves incubation of the pancreas with picric acid whereas frozen sections are simply air-dried or dipped in acetone before incubation with antibody B6. The qualitative difference in reaction pattern indicates that the method of pancreatic fixation can dramatically alter the interpretation of binding of even a single antibody. Such differences in reactivity indicate that the two techniques are not equivalent

and may contribute to differences in clinical assays using the two methods.

Drs Bottazzo and co-workers have described IgM antibodies in patients with type I diabetes and other individuals which bind to A cells on frozen sections⁵. Antibody B6 is an IgM monoclonal antibody which, on frozen sections, binds to A cells. As patients with type I diabetes express a series of anti-islet antibodies, the production of monoclonal antibodies with these specificities should now be feasible using the techniques which resulted in the generation of hybridoma B6. The ability to produce human monoclonal autoantibodies by simply fusing circulating lymphocytes provides a powerful tool for studying the autoimmune phenomenon of type I diabetes mellitus.

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A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor; partial characterization of the receptor

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T-cell growth factor (TCGF or interleukin-2) is an inducible glycoprotein hormone of molecular weight 15,000 (ref. 1) synthesized and secreted by T lymphocytes following activation with antigen or mitogen^{2,3}. TCGF is required for proliferation and expansion of T cells following antigen encounter^{4,5} and to maintain them in long-term culture *in vitro*^{6–9}. Full expression of the human immune response requires both the induction of TCGF synthesis and the formation of specific TCGF membrane receptors^{10,11}. Monoclonal antibodies binding TCGF have been prepared^{12,13}. In contrast, antibodies specific for the TCGF membrane receptor have not been identified, nor has the receptor been characterized. We have prepared a monoclonal antibody, termed anti-Tac^{14,15}, which appears to bind to the human membrane receptor for TCGF. In support of this, we now demonstrate that anti-Tac suppresses TCGF induced proliferation of T cells and blocks binding of radiolabelled TCGF to cells from a cloned human continuous T-cell line. Also we have partially purified and characterized the putative TCGF receptor. This receptor is a glycoprotein with a molecular weight (M_r) of 47,000–53,000.

Monoclonal anti-Tac was used in both ascites and Protein A-Sepharose purified¹⁶ forms. Two human continuous T-cell (CTC) lines, CTC-2 and HUT-102B2, were used in these studies. CTC-2 is TCGF dependent and derived from the peripheral blood of a patient with a nonleukaemic cutaneous T-cell lymphoma. CTC-2 is a subclone of CTC-1 and has been in culture for more than 2 yrs. HUT-102B2 is a cloned CTC line established from a lymph node of the same patient¹⁷. In contrast to CTC-2, HUT-102B2 constitutively produces sufficient endogenous TCGF so that exogenous TCGF is not required. Cells from this clone express membrane TCGF receptors¹⁸. As recently reported by Poiesz and co-workers¹⁹, HUT-102B2 cells contain and shed type C retrovirus. Cells from both CTC lines formed rosettes with sheep erythrocytes, reacted with OKT3 monoclonal antibody (a pan-T-cell-specific reagent)²⁰, and did not exhibit detectable surface immunoglobulin or Epstein-Barr nuclear antigen.

We initially looked at the effect of anti-Tac on TCGF-induced proliferation of CTC-2 cells. As shown in Fig. 1a, anti-Tac

ascites markedly inhibited TCGF-induced ³H-thymidine incorporation at final dilutions of 10⁻²–10⁻⁴, whereas equivalent concentrations of control ascites had no significant effect. Purified anti-Tac antibody also inhibited proliferation of CTC-2 cells, but purified monoclonal anti-Ia, though binding to these cells, had no effect (Fig. 1b). In agreement with these data, G. Bonnard and co-workers have independently demonstrated anti-Tac inhibition of TCGF induced proliferation of other human CTC lines (personal communication). In contrast, anti-Tac did not inhibit the growth of TCGF independent T-cell lines (CEM, HUT-78) or Epstein-Barr virus-transformed B-cell lines including a B-cell line derived from the same patient as the CTC lines.

All these experiments were performed in the absence of complement and anti-Tac did not produce cell death measured by short-term (4 h) ⁵¹Cr-release assays or long-term (24–48 h) supravital dye exclusion²¹ studies. In addition, the inhibitory effect of anti-Tac on proliferation of CTC-2 cells was reversible. CTC-2 cells precultured for 18 h with inhibitory concentrations of anti-Tac (10⁻³ dilution of ascites) incorporated thymidine in amounts equivalent to untreated cells following removal of cell-associated antibody by thorough washing and stimulation with TCGF.

We next investigated whether anti-Tac inhibited TCGF-induced proliferation by blocking the interaction of TCGF with its membrane receptor. ³H-TCGF was prepared by phytohaemagglutinin induction of TCGF synthesis in a cloned human T leukaemia cell line (JURKAT)²² in the presence of ³H-lysine and ³H-leucine. Radiolabelled TCGF produced in the absence of serum was purified by ultrafiltration and molecular sieve chromatography as previously described¹⁰, and produced a single spot when evaluated by two-dimensional gel electrophoresis. As shown in Fig. 2, preincubation of HUT-102B2 cells with anti-Tac at concentrations greater than 3 µg ml⁻¹ produced nearly complete blockade of purified ³H-TCGF binding in experiments performed at 37 °C. In contrast, while binding to these cells, similar amounts of monoclonal anti-Ia did not alter ³H-TCGF binding. The dose-response relationship

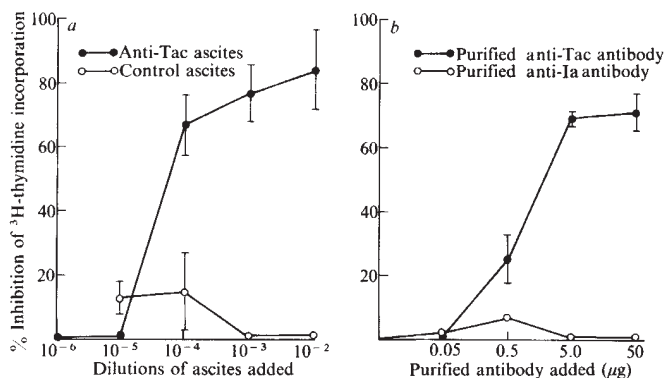


Fig. 1 Anti-Tac blocks TCGF induced proliferation of T cells from a human CTC line. CTC-2 cells were washed four times in a balanced salt solution and suspended at 0.5×10^6 cells per ml in RPMI 1640–10% heat-inactivated fetal calf serum. Aliquots (0.2 ml) of this cell suspension were distributed into wells of flat-bottom 96 well microtitre plates and preincubated with varying concentrations of antibodies for 30 min at room temperature. *a*, Data obtained with dilutions of heat-inactivated anti-Tac and control ascites (SPQC 11, from Dr M. Potter); *b*, data obtained with Protein A-Sepharose-purified anti-Tac and DEAE-purified monoclonal anti-Ia antibodies. Crude TCGF was subsequently added at a final concentration of 10% v/v. Triplicate cultures were incubated for 48 h at 37 °C in a humidified atmosphere containing 5% CO₂. Four hours before the end of culture, 1.0 µCi of ³H-methyl-thymidine was added to each well followed by transfer of cells onto fibreglass filters with a multiple channel automated cell collector. Cell-associated radioactivity was measured by liquid scintillation. Data are expressed as % inhibition of the response obtained in cultures receiving TCGF alone ($28,288 \pm 784$ c.p.m.).

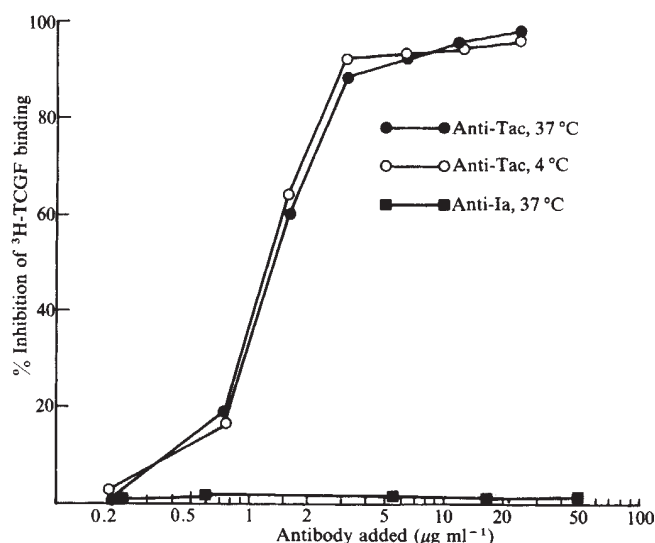


Fig. 2 Anti-Tac blocks the binding of ^3H -TCGF to HUT-102B2 cells. HUT-102B2 cells were washed three times in RPMI 1640 and resuspended at 1.1×10^7 cells per ml in RPMI 1640 containing 25 mM HEPES and 2 mg per ml bovine serum albumin. Cells and varying amounts of purified anti-Tac or anti-Ia antibodies were incubated together for 90 min at 37 or 4°C in a rotating water bath. Following this preincubation, 50 pmol of purified ^3H -Leu, ^3H -Lys TCGF were added and the reaction was continued for an additional 20 min at 37°C or 60 min at 4°C. Cells were then pelleted, resuspended in 100 μl of RPMI 1640, and centrifuged (9,000g for 90 s) through cushions of 84% silicone and 16% paraffin oil to remove unbound ^3H -TCGF. Microfuge tube tips were excised, placed in liquid scintillation vials, and the cells solubilized by addition of 100 μl 1% SDS and 3 ml of liquid scintillation mixture followed by measurement of cell-associated radioactivity. Each experiment was performed in duplicate. Data shown are from two different experiments. A third experiment performed at 37°C produced similar results.

for anti-Tac inhibition of TCGF-induced CTC proliferation and blockade of TCGF binding were similar in several experiments. As shown in Fig. 2, anti-Tac also inhibited ^3H -TCGF binding at 4°C. These data argue that anti-Tac inhibition of TCGF binding is not simply secondary to receptor capping or co-capping with subsequent receptor endocytosis or shedding, because these rearrangements are largely inhibited at 4°C (ref. 23).

Studies of ^{125}I -anti-Tac binding to HUT-102B2 cells at 20°C revealed half saturation of available receptor sites within 3.75 min of anti-Tac addition. Next, ^{125}I -anti-Tac was incubated with HUT-102B2 cells at 4°C in the presence of 0.1% sodium azide to block cap formation and energy-dependent endocytosis. After 1 h, steady-state binding was achieved. Excess unlabelled antibody was then added and reversible binding was demonstrated as 90% of the bound radiolabelled anti-Tac molecules were shown to dissociate. Glutaraldehyde fixed HUT-102B2 cells bound ^{125}I -anti-Tac in amounts slightly less than non-fixed cells. Preliminary Scatchard analysis²⁴ of anti-Tac binding with HUT-102B2 cells indicates $\sim 100,000$ high-affinity receptors per cell. This receptor number is 7- to 8-fold higher than the number of TCGF receptors reported by Robb *et al.*¹⁰. This difference may in part be accounted for by different experimental binding conditions and slightly different cell lines (our HUT-102B2 line is not 6-thioguanine resistant in contrast to that of Robb and co-workers). Anti-Tac had a K_d of $\sim 10^{-9}$ mol l^{-1} suggesting a much lower affinity than that reported for TCGF ($K_d \sim 10^{-12}$ mol l^{-1})¹⁰. Thus, the preincubation with anti-Tac before addition of ^3H -TCGF may have been critical to our ability to perform the successful competitive binding experiment (Fig. 2).

Finally, we have partially purified and characterized the membrane receptor recognized by anti-Tac. SDS-polyacrylamide

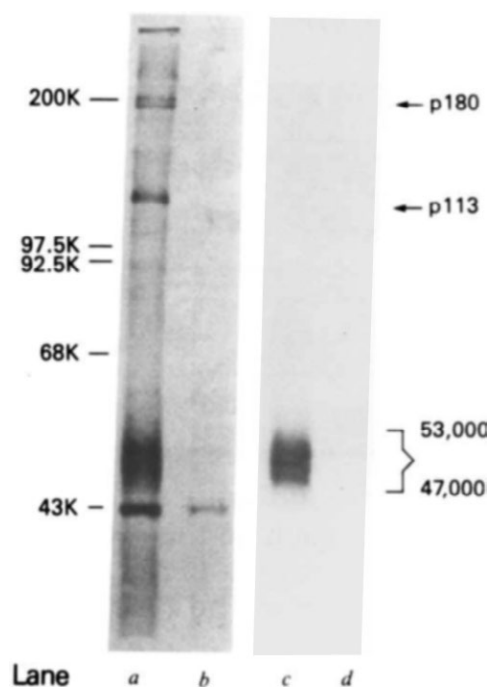


Fig. 3 Purification of the anti-Tac membrane receptor. Five million HUT-102B2 cells were washed twice in a balanced salt solution and suspended in 5 ml of methionine-free Selectamine medium (Gibco) containing 5% dialysed fetal calf serum. 0.3 mCi of NEN translation grade ^{35}S -methionine (>800 Ci mmol^{-1}) was added and the cells were cultured overnight at 37°C. Cells were washed once and extracted in 0.14 M NaCl, 10 mM Tris pH 7.5, 1% Triton X-100, and 100 μg ml^{-1} phenylmethylsulphonyl fluoride (PMSF) for 30 min at 4°C. The supernatant of a 60-min spin at maximum speed was then immunoprecipitated as follows. The supernatant was cleared once with 5 μl of control ascites (NS1, a non-secreting ascites provided by Dr Jay Berzofsky, NIH), 5 μl of RPC5 (a control IgG2a-k antibody, Litton Bionetics), and 50 μl of 10% w/v formaldehyde fixed Cowan I strain staphylococci and then once with 50 μl of the staphylococcal organisms alone. The supernatant was immunoprecipitated with 2-3 μg of protein A sepharose purified anti-Tac or UPC10 (a second control IgG2a-k antibody, Litton Bionetics) for 20 min at room temperature followed by a 20 min incubation with 20 μl of Cowan I strain staphylococci at room temperature. The immunoprecipitate was resuspended in a buffer containing 0.1% SDS, 0.5% NP40, 0.2% deoxycholate, 100 μg ml^{-1} PMSF and washed twice through the same buffer containing 1M sucrose. Final pellets were boiled in 1% SDS in the presence or absence of 0.1M dithiothreitol and analysed on 7.5% discontinuous SDS-polyacrylamide gels. Gels were intensified and fluorographed for 24 to 48 h at -195°C . Analysis under reducing conditions is shown. *a* and *b* show the electrophoretic patterns obtained following immunoprecipitation of ^{35}S -methionine incorporated cells with anti-Tac and UPC10, respectively. The bands at 200,000 (200K) and 43,000 (43K) co-migrate with myosin and actin, respectively. Migration of molecular weight standards is shown on the left. The locations of p180, p113, and p50 are indicated on the right. *c* and *d* represent electrophoretic patterns obtained after immunoprecipitating surface-labelled cells with anti-Tac and UPC10, respectively. Approximately $5-10 \times 10^6$ cells were washed and suspended in 100 μl of a balanced salt solution and 1 mCi carrier-free ^{125}I was added. 4 μl lactoperoxidase solution (1U per 10 μl) and 7.5 μl H_2O_2 solution (0.03%) were then added and cells incubated for 4 min at room temperature. An additional 2 μl of lactoperoxidase solution and 7.5 μl H_2O_2 were added and cells incubated for an additional 4 min. Cells were then washed in a balanced salt solution and immunoprecipitated as described above.

gel electrophoretic analysis of this receptor from HUT-102B2 cells is shown in Fig. 3. In both reducing (shown) and nonreducing (not shown) conditions, two major proteins with molecular weights of approximately 113,000 and 47,000-53,000 were precipitated by anti-Tac antibodies from cells internally labelled

with ^{35}S -methionine (a). A third minor band with an M_r of approximately 180,000 was also identified. For convenience, we will denote these bands p113, p50, and p180, respectively. None of these bands was identified in immunoprecipitations using control UPC10 IgG2a-k antibodies (b). Further, similar bands have been found in immunoprecipitations performed on phytohaemagglutinin stimulated peripheral blood lymphocytes from a normal individual (data not shown), thereby providing evidence that none are related solely to viral products. To determine whether any of these proteins are located on the outer cell membrane, similar anti-Tac and UPC10 immunoprecipitations were performed using cells that were surface iodinated with the lactoperoxidase method (c and d). Only p50 was immunoprecipitated with anti-Tac. We therefore hypothesize that this protein represents the actual binding site for anti-Tac. This protein can be identified in immunoprecipitations from cells incorporated with ^3H -D-glucosamine (Fig. 4), thus demonstrating that it is a glycoprotein, as is usually the case for membrane receptors. The roles of p113 and p180 are unknown. One or both may be part of a theoretical receptor complex, and therefore coimmunoprecipitated because of strong hydrophobic interactions with p50. It is also possible that neither has any functional relation to the receptor identified by anti-Tac, but rather that they contain antigenic determinants that result in their recognition by anti-Tac.

In summary, we have prepared a monoclonal antibody which blocks the binding of purified ^3H -TCGF to human CTC cells and inhibits TCGF induced proliferation of CTC cells, and which we hypothesize binds to the TCGF receptor. The receptor recognized by the antibody is a glycoprotein of molecular weight $\sim 47,000$ – $53,000$. In recent studies with human peripheral blood T cells, we have also found that anti-Tac inhibits ($>80\%$) soluble or alloantigen induced T-cell proliferation as well as the generation of cytotoxic T cells in allogeneic cell cocultures. Further, anti-Tac suppresses immunoglobulin production in cultures of peripheral blood lymphocytes stimulated with pokeweed mitogen, a T-cell dependent polyclonal activator. Thus, anti-Tac may permit not only further characterization of

the human TCGF receptor but in addition may allow analysis of the site(s) and mechanism of TCGF action in both the cellular and humoral phases of the human immune response.

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Luteinizing hormone release from dissociated pituitary cells by dimerization of occupied LHRH receptors

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Studies of luteinizing hormone-releasing hormone (LHRH) have revealed several structural requirements for its biological activity¹. Shortening of the decapeptide leads to loss of activity; positions 1, 6 and 10 are regarded as important in that they have a high affinity for the hormone receptor, and positions 2 and 3 (His-Trp) are thought to comprise the active centre of the molecule (replacement of His or Trp produces the most effective antagonists). Using a potent agonist analogue of the decapeptide, $[\text{Glu-His-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-NH}_2]$, coupled to ferritin, we have demonstrated previously the binding, aggregation and internalization of bound hormone using dissociated pituitary cells². Similar processes occur for other biologically active peptides, for example, insulin³ and epidermal growth factor (EGF)⁴. Using a pure antagonist in the simpler LHRH system we now show that the N-terminal portion of the molecule is important for receptor aggregation and that dimerization of occupied LHRH receptors is a necessary early stage in the release of luteinizing hormone.

The octapeptide, Z-Gln-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NH₂, is an effective inhibitor of LHRH both *in vitro* and *in vivo*⁵. We prepared the 6-D-Lys analogue of this peptide to allow easy substitution at a central position in the molecule;

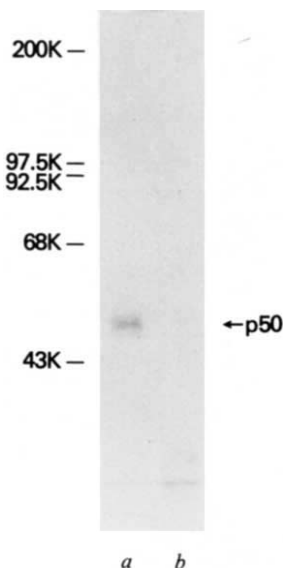


Fig. 4 The anti-Tac membrane receptor is a glycoprotein. 5×10^6 HUT-102B2 cells were washed once in a balanced salt solution and resuspended in RPMI media containing 5% fetal calf serum. Two hundred microcuries of D-[1,6- ^3H]-glucosamine HCl (NEN, $32.5 \text{ Ci mmol}^{-1}$) were then added and cells incubated for 4 h at 37°C . Cells were washed once in balanced salt solution and then extracted with 0.14 M NaCl, 10 mM Tris-HCl pH 7.5, 1% Triton X-100, and $100 \mu\text{g ml}^{-1}$ PMSF for 30 min on ice. The extracts were then immunoprecipitated with anti-Tac (a) or UPC10 (lane b) as described in Fig. 3 legend, and then electrophoresed on 7.5% SDS gels and autoradiographed. Molecular weight markers are indicated on the left.

Table 1 Secretion of luteinizing hormone from porcine pituitary cells in culture

Peptide	Antiserum	LH released
LHRH (10^{-9} M)		185 $\pm$ 24
LHRH (10^{-8} M)		300 $\pm$ 34
Inhibitor (10^{-9} M)		29 $\pm$ 5
Inhibitor (10^{-6} M)		49 $\pm$ 5
LHRH (10^{-9} M) + inhibitor (10^{-6} M)		35 $\pm$ 12
Inhibitor (10^{-8} M)	Rabbit anti-inhibitor (1:1,000 dilution)	215 $\pm$ 36
Inhibitor (10^{-6} M)	Rabbit anti-inhibitor (1:1,000 dilution)	112 $\pm$ 16
Inhibitor (10^{-8} M)	Rabbit anti-D-Lys agonist (1:1,000 dilution)	29 $\pm$ 8
LHRH (10^{-8} M)	Rabbit anti-inhibitor (1:1,000 dilution)	361 $\pm$ 51

Cells were incubated at 37 °C for 24 h after dissociation at 4×10^6 cells per 2.5-cm diameter Petri dish; before each experimental incubation they were washed three times in serum-free medium. The cells were then incubated in 1 ml medium for 30 min with additions as shown. Values are $\text{ng ml}^{-1} \pm \text{s.e.m.}$ luteinizing hormone.

this in turn would allow the formation of active macromolecular conjugates with the agonist². The peptide was prepared from the protected heptapeptide described previously² (H-Trp-Ser-Tyr(Bu^t)-D-Lys(BOC)-Leu-Arg-Pro-NH₂). This was coupled, in dimethylformamide, with the activated derivative Z-Gln-OCF₃, and the product purified by chromatography on Sephadex LH-20 in methanol. The side chain protecting groups were removed with trifluoroacetic acid/water (9:1) and the peptide was isolated by chromatography on Sephadex G-50 in acetic acid/water (1:4). Analysis of an acid hydrolysate showed good amino acid ratios and the peptide was revealed as a single spot by high voltage electrophoresis at pH 2.1, 6.5 and 8.9 using ninhydrin, Ehrlich's and Sakaguchi visualization reagents and by TLC on silica gel plates in *n*-butanol/acetic acid/water (4:1:5).

The pure peptide was then coupled through the side chain of the central Lys residue to bovine serum albumin (BSA) using 1-ethyl-3(3-dimethylaminopropyl)carbodiimide. Antisera to the peptide were raised in rabbits by injection of conjugate (1.0 mg) in Freund's complete adjuvant subcutaneously (s.c.), followed by two booster injections of conjugate in saline at intervals of 4 weeks. The antisera were able to bind iodine-labelled LHRH, indicating that there were features common to the hormone and antagonist.

Dissociated porcine pituitary cells were prepared as described previously⁶ and luteinizing hormone (LH) release measured by radioimmunoassay for the various treatments (Table 1). Clearly, the 6-D-Lys analogue of the antagonist⁵ retained similar activity *in vitro* as gonadotropin release by the native hormone was suppressed completely at concentrations 100 times that of the agonist. The analogue alone gave no gonadotropin release at concentrations up to 10^{-6} M, indicating that the occupied receptor complex lacked the structure required for a biological

response. However, the presence of antibodies converted this antagonist complex into a functional agonist by aggregating occupied receptors as a first stage. Lower levels of gonadotropin were secreted at higher concentrations of peptide, presumably because of extensive binding of antibody by free peptide in solution. The specificity of the effect was demonstrated by the fact that the antiserum raised against the peptide having [Glu-His instead of Z-Gln was unable to evoke a response. Although this indicated that antibody binding occurred through the N-terminal region, the anti-inhibitor antiserum may have multiple specificity and could also contain different immunoglobulin isotypes. Thus, although aggregation is involved, its scale and specificity is unclear.

From the rabbit antiserum to the inhibitory peptide, we prepared pure IgG having specificity for the N-terminus of the peptide (for experimental details see Table 2). The purified specific IgG and Fab proteins were then used to study LH release from pituitary cells (Table 2).

For this particular cell preparation, LHRH at 10^{-7} M stimulated LH release 3–4-fold. The inhibitor alone was ineffective at 10^{-9} M but in conjunction with purified specific IgG at $\sim 10^{-11}$ M, LH release occurred. That this required divalency was apparent from the inability of similar amounts of Fab fragment to evoke similar effects and by the ability of goat anti-rabbit anti-serum to induce the Fab-antagonist complex to stimulate LH release. The effect of all agents together (Table 2) indicated that LH release was only mediated via the LHRH receptor.

Previously, we have observed aggregation and internalization of bound LHRH² and it has been shown that receptor aggregation is essential for the action of some polypeptide hormones. Thus, antibodies to the insulin receptor produced insulin-like responses directly^{7,8} and a degraded EGF analogue became

Table 2 Sequential experiments with porcine pituitary cells in culture showing LH release for each treatment period.

Control period	Period 1	Period 2
25.0 $\pm$ 2.4	LHRH (10^{-7} M)	
30.3 $\pm$ 4.2	Inhibitor (10^{-9} M)	
30.5 $\pm$ 1.8	Inhibitor (10^{-9} M) + 1.3 ng ml^{-1} IgG	
20.0 $\pm$ 4.7	Inhibitor (10^{-9} M) + 0.13 ng ml^{-1} IgG	
30.5 $\pm$ 3.73	Inhibitor (10^{-9} M) + 0.8 ng ml^{-1} Fab	
23.6 $\pm$ 2.8	Inhibitor (10^{-9} M) + 0.8 ng ml^{-1} Fab + LHRH (10^{-7} M)	
		Inhibitor (10^{-9} M) + 0.8 ng ml^{-1} Fab + GaRS (1:20)
		Inhibitor (10^{-9} M) + 0.8 ng ml^{-1} Fab + LHRH (10^{-7} M) + GaRS (1:20)

Pituitary cells were prepared and plated out as described in Table 1. Between each 30-min incubation period, the cells were rinsed with serum-free medium for three 1-min periods. Values are $\text{ng ml}^{-1} \pm \text{s.e.m.}$ luteinizing hormone. The goat anti-rabbit serum (GaRS) had no significant effect on LH release when used alone at 1:20 dilution. The purified specific IgG and Fab proteins used in the study were prepared as follows.

Rabbit antiserum (1 ml) to the inhibitory peptide, diluted with phosphate-buffered saline (PBS), was recycled through an affinity column comprising 2 ml Sepharose coupled to 20 mg of the C-terminal peptide-sequence (Leu-Arg-Pro-NH₂) at 4 °C for 48 h. The non-retarded serum solution was recycled through a 5 ml column of Sepharose-Protein A (Pharmacia) for 24 h. The column was washed with 5 vol PBS, eluted with 5 ml 0.1 M citrate buffer, pH 3.5 and the eluate neutralized immediately with 0.5 ml M Tris-HCl buffer, pH 8.0. The lyophilized eluate was taken up into water and applied to a column of Ultrogel ACA 34 (60 $\times$ 0.9 cm) in 0.05 M ammonium bicarbonate. The fractions from the single peak in the precalibrated IgG position (2.6 mg) were combined and lyophilized. Half the sample was taken up into 200 μl buffer (0.1 M phosphate pH 7.0, 0.01 M cysteine, 0.002 M EDTA) and treated with 15 μg papain at 37 °C for 16 h. In the subsequent Ultrogel chromatography designed to obtain Fab fragments, we observed no intact IgG.

mitogenic when aggregated by antibody⁹. However, for these larger molecules, it is possible that the antisera have multiple specificity; the simpler octapeptide used here allows clearer definition of the effects.

Since our initial report of the conversion of a pure antagonist to agonist¹⁰, we have shown that a different antagonist when converted to a dimer, could be activated using anti-serum¹¹, thus confirming the phenomenon. Here, use of purified immunoglobulin and peptide monomer allows firm conclusions to be made concerning the nature of the hormone-receptor interactions.

The high specificity of the hormone for the receptor presumably involves several amino acids, and the antigenic determinants of proteins and polypeptides have also been shown to encompass several amino acids¹². Thus, it is unlikely that residues contained in the antigen-binding site are available for receptor binding at the same time and vice versa; the logical conclusion is that the octapeptide cannot bind more than one antibody molecule concurrent with the receptor site being occupied by this inhibitor. The IgG preparation, which is a

divalent protein specific to the peptide N-terminus, can produce a biological response from an inactive hormone complex. This indicates that formation of an occupied receptor dimer (and no greater aggregation is required) by lateral movement through the membrane is a necessary step in the response pathway after binding of hormone to the receptor. Accepting that the octapeptide is only large enough to bind to the receptor and to one antibody molecule, it follows that the C-terminal region must have a dominant role in binding to the receptor, and this is common to both agonist and antagonist. That the anti-antagonist antibody recognizes the N-terminal region is supported by the fact that the anti-agonist antibody (Table 1) was unable to induce LH release, thus it is proposed that the $\square$ Glu-His-Trp-Ser sequence is important in the dimerization of occupied receptors. We conclude, therefore, that this small peptide hormone is able to exert an effect on a normal cell population by having a sequence allowing specific binding, followed by a second sequence that causes dimerisation of the receptors, thus leading to biological expression by an as yet undefined series of stages.

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Tumour promotion by TCDD in skin of HRS/J hairless mice

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) serves as the prototype for a group of environmental toxicants, the halogenated aromatic hydrocarbons¹⁻³, which evoke a characteristic pattern of acute toxic and biochemical responses, and which produce these effects by stereospecific and reversible binding to a soluble protein receptor^{2,4,5}. The chronic administration of TCDD⁶⁻⁹ (and several other halogenated aromatic hydrocarbons)¹⁰⁻¹³ to rats and mice has produced an increased incidence of hepatocellular carcinomas as well as tumours in other tissues. However, because TCDD is not a mutagen^{14,15} and does not bind appreciably to DNA *in vivo*¹⁶, it has been suggested that this compound is not a complete carcinogen, but rather acts as a tumour promoter, enhancing neoplastic expression in already initiated cells^{17,18}. TCDD has been shown to be a potent tumour promoter in a two-stage model of carcinogenesis in rat liver¹⁹, but has proven ineffective in the classical model in mouse skin (using CD-1 and Swiss-Webster mice)^{20,21}. We have recently shown that TCDD produces epidermal hyperplasia and hyperkeratosis, and squamous metaplasia of the sebaceous glands in mice bearing the recessive trait, *hairless* (*hr*), but causes no changes in the skin of mice which are wild type or heterozygous at this locus²². Here we examine the capacity of TCDD to promote tumour formation in the skin of HRS/J hairless (*hr/hr*) mice and their congenic, haired (*hr/+*) littermates. Following the application of an initiating dose of a carcinogen, repeated skin application of TCDD produced papillomas in *hr/hr* mice but not *hr/+* mice. In HRS/J hairless mice, tumour promotion by TCDD elicits the same incidence and multiplicity of skin papillomas as does the classical tumour promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), but at 1/100th the dose.

HRS/J is an inbred strain of mice segregating for the *hr* locus²³; haired mice (*hr/+*) and hairless mice (*hr/hr*) are genetically identical except at the *hr* locus and closely linked loci on

Table 1 Comparison of TCDD and TPA as tumour promoters in HRS/J hairless mice

Initiation	Promotion	At 20 weeks			
		Tumour incidence (surviving mice with tumours/surviving mice)	Tumour multiplicity (average no. of papillomas/ surviving mice)		
MNNG	Acetone	1/19	5%	0.05	
MNNG	TCDD	3.75 ng*	11/20	55%	0.7
MNNG	TCDD	7.5 ng	13/17	77%	1.5
MNNG	TCDD	15 ng	10/10	100%	4.0
MNNG	TCDD	30 ng	15/19	79%	1.6
Acetone	TCDD	30 ng	0/19	0%	0
MNNG	TPA	1 μg	5/19	26%	0.4
MNNG	TPA	3 μg	13/18	72%	1.6

HRS/J hairless female mice, 7 weeks old, were administered a single dose of MNNG (5 µmol in 50 µl of acetone) or the solvent alone applied to the dorsal skin, and then treated twice weekly with various doses of TCDD or TPA (in 50 µl of acetone) for 20 weeks. There were initially 20 mice per group and the survival was 85% or more in all groups except group 4 (TCDD 15 ng). Ten animals died in this group after 15 weeks of promotion, from dehydration coincident with being changed to a new type of cage. These deaths did not alter the tumour incidence or tumour multiplicity, that is, at 15 compared with 16 weeks.

* Dose of compound per mouse, given twice weekly.

chromosome 14. Initiation of the skin of HRS/J (*hr/+*) haired mice with dimethylbenz(a)anthracene (DMBA 0.2 µmol) and subsequent twice-weekly topical application of the classical tumour promoter TPA, 2 µg per mouse, produced skin papillomas (Fig. 1a). Administration of DMBA alone or repeated application of TPA without prior initiation produced no tumours. Compared with other mouse strains²⁴, HRS/J haired mice are relatively sensitive to this regimen of DMBA/TPA: by 11 weeks of promotion, 100% of the mice developed papillomas, and by 14 weeks the tumour multiplicity (number of papillomas/number of surviving mice) was 9.2 papillomas per mouse. Following initiation with DMBA, repeated topical administration of TCDD (20 ng per mouse twice weekly for 8 weeks, then 50 ng per mouse twice weekly) failed to produce any papillomas over the 25-week course of the experiment.

In contrast, HRS/J (*hr/hr*) hairless mice, initiated with DMBA and treated with the same regimen of TCDD, did develop papillomas (Fig. 1b). After 25 weeks of TCDD

promotion, 15 of 19 surviving mice (79%) had one or more skin tumours, with an average multiplicity of 1.4 tumours per mouse. Administration of DMBA alone produced a single papilloma, and in the absence of initiation, TCDD produced no papillomas. The time course of tumour incidence and tumour multiplicity produced by promotion with TCDD (20 ng per mouse, twice weekly) and TPA (2 µg per mouse, twice weekly) were very similar (Fig. 1c). Thus, TPA promotes tumour formation in the skin of both HRS/J haired and hairless mice, while TCDD is effective only in *hr/hr* mice.

A further comparison of TPA and TCDD is shown in Table 1. HRS/J hairless mice were initiated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and treated twice weekly with various doses of TCDD or TPA. TCDD produced a dose-related increase in the incidence and multiplicity of skin papillomas at doses of 3.75, 7.5 and 15 ng per mouse. At the highest dose tested, 30 ng per mouse, TCDD produced greater toxicity and was less effective as a tumour promoter. Comparable tumour promotion was produced by twice-weekly administration of TCDD at 7.5–15 ng per mouse ($2.3\text{--}4.6 \times 10^{-11}$ mol) and TPA at 3 µg per mouse (4.8×10^{-9} mol); thus, TCDD seems to be ~100 times more potent than TPA on a molar basis. However, one cannot formally compare the relative potencies of these compounds unless they act by a similar mechanism and produce the same maximum response with parallel log dose-response curves.

We examined the capacity of several halogenated aromatic hydrocarbon congeners and commercial mixtures of halobiphenyl isomers to promote tumour formation in the skin of HRS/J hairless mice (Table 2). 2,3,7,8-Tetrachlorodibenzofuran (TCDBF) and 3,4,5,3',4',5'-hexabromobiphenyl, both approximate isostereomers of TCDD which bind to the cytosol receptor and evoke a number of receptor-mediated responses including induction of microsomal monooxygenase activity^{5,25}, terminal differentiation of XB cells in culture²⁶ and hyperplasia and hyperkeratosis in the skin of hairless mice²², are effective tumour promoters. TCDBF (1 µg per mouse, twice weekly) produced a greater tumour multiplicity than a near-optimally effective regimen of TCDD, and the TCDBF-treated mice showed less toxicity and better weight gain. The relatively rapid metabolism of TCDBF in the mouse²⁷ may permit attainment of a high local skin concentration of the compound without systemic accumulation and toxicity. 2,7-Dichlorodibenzo-*p*-dioxin and 2,4,5,2',4',5'-hexabromobiphenyl, congeners which

neither bind to the cytosol receptor nor produce the characteristic biochemical and toxic effects of TCDD^{22,25}, were inactive as tumour promoters at the doses tested. Aroclor 1254 (1 mg per mouse twice weekly), a commercial mixture of chlorinated biphenyl isomers which has been widely used in industry in capacitors and transformers, as a lubricant and heat transfer fluid, slightly enhanced the incidence of skin tumours in MNNG-initiated mice, but this effect was not statistically significant. In contrast, Firemaster FF-1, a commercial mixture of brominated biphenyl isomers used as a flame retardant²⁸, was an effective tumour promoter, but the dose regimen used was toxic and produced severe hepatomegaly and hepatic porphyria.

Several aspects of these experiments require further comment. First, the epidermal and systemic effects produced by repeated skin painting with TPA and TCDD are in sharp contrast. In HRS/J haired and hairless mice, TPA produces an acute inflammation and hyperplasia of the epidermis, with little systemic toxicity. TCDD produces no gross or histological changes in the skin of *hr/+* mice²². Hairless mice, repeatedly treated with TCDD, develop a scaly, shiny and taut appearance to their skin, and histologically one observes epidermal hyperplasia and hyperkeratosis, squamous metaplasia of the sebaceous glands, and accumulation of keratinaceous material in the dermal cysts which are normally found in *hr/hr* mice. In contrast to TPA, TCDD produces no acute inflammatory response in the skin, neither oedema nor leukocyte infiltration²². TCDD and other halogenated aromatic hydrocarbons produce several dose-related systemic lesions in both *hr/hr* and *hr/+* mice: reduced weight gain or, at high doses, weight loss, loss of cutaneous and abdominal fat, thymic atrophy and liver enlargement and parenchymal cell changes²⁹. Hairless mice repeatedly administered high doses of TCDD have skin which is easily damaged and may develop abscesses about the face and in the preputial and rectal glands. The animals assume a humped stature, with the back arched above the shoulders, and they move about poorly.

Second, the incidence and multiplicity of tumours produced by initiation and TPA promotion are less in HRS/J hairless mice than in haired mice, as noted previously by Giovanella *et al.*³⁰ and seen in Fig. 1b, c. Thus, the relatively low tumour multiplicity produced by TCDD and congeners in HRS/J *hr/hr* mice is a constraint of the animal model, and not a reflection of a poor promoting efficacy of these compounds.

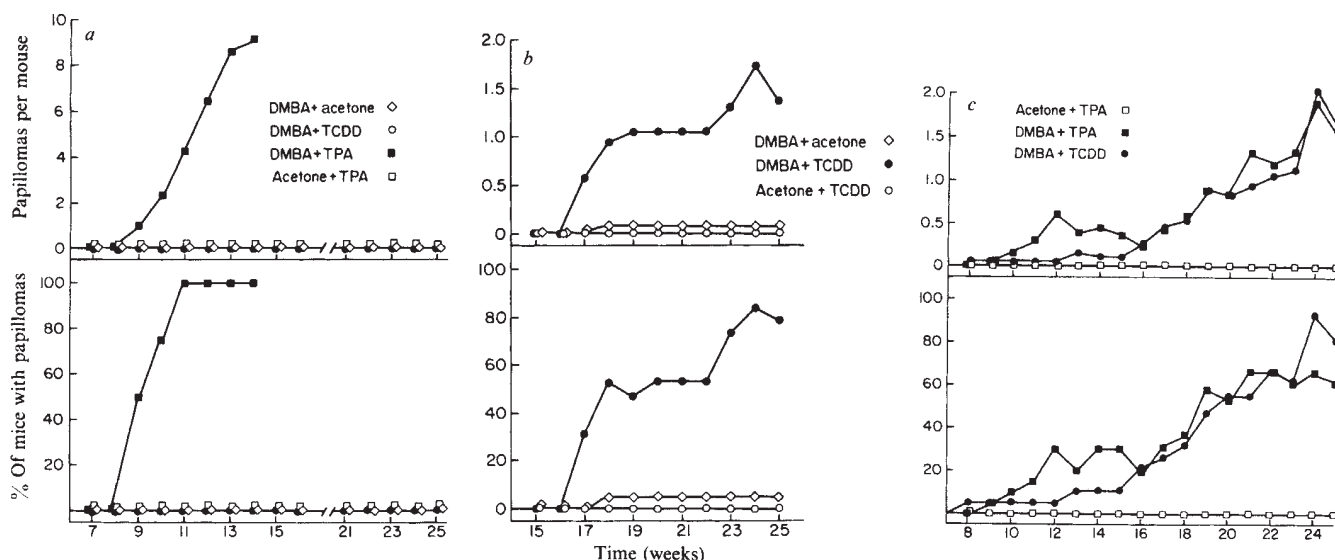


Fig. 1 Tumour promotion by TPA and TCDD in the skin of HRS/J *hr/+* and *hr/hr* mice. *a*, Haired (*hr/+*) mice were shaven, initiated with a single dose of DMBA (0.2 µmol) applied to the skin, or with solvent (acetone), and then painted twice weekly with TPA (2 µg per mouse), TCDD (50 ng per mouse for 8 weeks, then 20 ng per mouse) or acetone. *b*, Hairless (*hr/hr*) mice: treatment regimens same as in *a*. *c*, Hairless (*hr/hr*) mice were administered DMBA (0.2 µmol) twice, 1 week apart, or the solvent, and then topically treated twice weekly with TPA (2 µg per mouse) or TCDD (20 ng per mouse). For experiments in *a*–*c*, each treatment group consisted of 20 female mice, and had 16 or more survivors at the end of the experiment. All compounds were dissolved in acetone: for initiation 100 µl of the acetone solution of DMBA was applied to *hr/+* and *hr/hr* mice, and for promotion 50 µl of acetone or acetone solutions were applied to *hr/hr* mice, and 100 µl of these solutions to *hr/+* mice.

Table 2 Tumour promotion by halogenated aromatic hydrocarbons in HRS/J hairless mice

Group	Initiation	Promotion	Dose (dose per mouse, twice weekly)	n	20 weeks		
					Tumour incidence (surviving mice with tumours/surviving mice)	Tumour incidence (surviving mice with tumours/surviving mice)	Tumour multiplicity (average no. of papillomas/surviving mice)
1	MNNG	Acetone		26	0/23	0%	0
2	Acetone	TCDD	50 ng, 5 weeks, then 20 ng	26	0/18	0%	0
3	MNNG	TCDD	50 ng, 5 weeks, then 20 ng	26	16/19	84%	1.6
4	MNNG	2,3,7,8-Tetrachlorodibenzofuran	1.0 µg	20	19/19	100%	4.9
5	Acetone	2,3,7,8-Tetrachlorodibenzofuran	1.0 µg	20	1/20	5%	0.05
6	MNNG	3,4,5,3',4',5'-Hexabromobiphenyl	20 µg	20	12/20	60%	1.5
7	Acetone	3,4,5,3',4',5'-Hexabromobiphenyl	20 µg	20	0/20	0%	0
8	MNNG	2,4,5,2',4',5'-Hexabromobiphenyl	20 µg	20	0/20	0%	0
9	Acetone	2,4,5,2',4',5'-Hexabromobiphenyl	20 µg	26	0/22	0%	0
10	MNNG	2,7-Dichlorodibenzo-p-dioxin	20 µg	20	0/19	0%	0
11	Acetone	2,7-Dichlorodibenzo-p-dioxin		20	0/20	0%	0
12	MNNG	Aroclor 1254	1 mg	20	4/19	21%	0.3
13	Acetone	Aroclor 1254	1 mg	20	0/19	0%	0
14	MNNG	Firemaster FF-1	2 mg, 5 weeks, then 1 mg	26	9/15	60%	2.0
15	Acetone	Firemaster FF-1	2 mg, 5 weeks, then 1 mg	20	1/16	6%	0.13

HRS/J hairless female mice, 8 weeks old, received a single administration of MNNG (5 µmol in 50 µl of acetone) or solvent alone applied to the skin and were then treated topically twice weekly with test compound dissolved in 50 µl of acetone for 20 weeks. Treatment with Firemaster FF-1 produced a significant mortality and the dose was reduced after 5 weeks. The appreciable mortality in other groups (2, 3 and 6) occurred largely in the first 6 weeks of the experiment, resulted primarily from dehydration and did not seem to be a result of the treatment regimen. Additional animals were added to these groups, so all groups consisted of 20 or 26 mice.

Third, skin tumours elicited by promotion with TCDD (and congeners) were often smaller (1–3 mm) and progressed more slowly than those elicited by promotion with TPA. Virtually all the tumours were keratinizing squamous cell papillomas, regardless of the promoter used. Finally, in all treatment groups (Fig. 1a–c), the survival rate was >80%. HRS/J mice harbour a murine leukaemia virus, and the expression of the recombinant polytropic virus and the incidence of lymphatic leukaemia are greater in *hr/hr* mice than *hr/+* mice^{31–33}. By 10 months of age, leukaemia develops in 45% of *hr/hr* mice and only 1% of the heterozygote *hr/+* mice³¹. In all experiments with *hr/hr* mice (Fig. 1b, c, Tables 1, 2), 0–15% of the animals developed leukaemia (as evidenced by enlarged spleen, thymus, lymph nodes and/or liver) which either resulted in their premature death or was detected at autopsy at the termination of the experiment. The incidence of leukaemia was not significantly different in any of the treatment groups, and seemed to be independent of exposure of the animals to a carcinogen or halogenated aromatic hydrocarbons.

TCDD and related halogenated aromatic hydrocarbons comprise a class of potent tumour promoters which seem to act by a mechanism that is distinct from that of TPA and its congeners. The epidermal hyperplasia²² and most probably the tumour promotion (as suggested by the structure–activity relationship in Table 2) produced in hairless mice by these halogenated aromatic hydrocarbons are mediated through the cytosol receptor. In contrast, the promoting activities of TPA, other phorbol and diterpene esters, and structurally unrelated compounds such as teleocidin and debromoaplysiatoxin^{34–37}, apparently act through another mechanisms.

Two aspects of this model of tumour promotion in the skin of hairless mice may prove especially useful. First, comparison of the pleiotropic responses produced by TCDD in the skin of congenic HRS/J *hr/hr* and *hr/+* mice may permit identification of the additional battery of genes expressed in *hr/hr* skin which are in some manner associated with promotion. Second, it may be determined if any of the biochemical effects produced by TPA and congeners and implicated in their mechanism of action¹⁸ are also common to the response produced by halogenated aromatic hydrocarbons.

Our model of tumour promotion by TCDD and congeners may be relevant to humans because the hyperplastic/metaplastic response produced in the skin of the mutant hairless mouse is similar to that evoked by these compounds in human skin.

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Surface sialic acid reduces attachment of metastatic tumour cells to collagen type IV and fibronectin

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Attachment of cells to substrate containing fibronectin, laminin and collagens has been shown to influence cell proliferation¹, differentiation^{2,3} and migration⁴, aspects of cellular behaviour known to be altered by malignant transformation. Recent studies on mutant cell lines displaying altered attachment to substrate and changes in cell-surface carbohydrate composition have provided evidence for the involvement of glycoconjugates in cell attachment⁵⁻⁷. Using this approach, we have now found that a non-metastatic wheat germ agglutinin-resistant (WGA^R) mutant of the highly metastatic murine tumour MDAY-D2 has a three- to fourfold reduction in neuraminidase-accessible cell-surface sialic acid which seems to allow enhanced attachment to basement membrane collagen type IV and fibronectin, but not to laminin. Furthermore, neuraminidase treatment of the WGA-sensitive metastatic tumour cells enhanced their attachment to collagen type IV and fibronectin, but not to laminin. Neuraminidase treatment had no effect on attachment of the WGA^R mutant cells to any of the substrates.

Yogeeswaran and Salk⁸ have recently shown that the metastatic capacity of a range of murine tumour cell lines is positively correlated with the degree of sialation of available galactose and *N*-acetylgalactosamine residues on cell-surface glycoconjugates. This correlation is supported by the finding that some WGA^R mutants of the B16 melanoma are non-metastatic⁹ and deficient in cell-surface sialic acid¹⁰. WGA binds preferentially to the oligosaccharide portions of glycoconjugates containing *N*-acetylglucosamine and sialic acid¹¹. The lectin must bind to the cell surface to exert its toxic effects and, as might be expected, some genotypic classes of WGA^R cells have less surface sialic acid and hence show decreased WGA binding¹². MDW40, the non-metastatic WGA^R mutant of MDAY-D2, also binds less WGA^{13,14} and shows a three- to fourfold reduction in neuraminidase-accessible sialic acid compared with the parental tumour cells and two WGA-sensitive (WGA^S) revertants designated W40M1 and W40M4 (Table 1). The highly metastatic WGA^S revertants are lines established from metastatic nodules found in the organs of mice injected with the WGA^R mutant. MDW40 cells have previously been shown to grow only at the subcutaneous site of injection where over time, one or a few cells become WGA sensitive and highly metastatic. The revertant tumour cells progressively dominate the primary tumour cell population and metastasize to distant organs^{14,15} (for example, W40M1 and W40M4). The origin and phenotypes of the tumour cell lines are summarized in Table 1. These observations are similar to those of Bramwell and Harris who have shown that some WGA^R tumour cell variants which are poorly tumorigenic revert at the site of inoculation to the WGA-sensitive and more malignant phenotype¹⁶.

Two WGA^R mutants of MDAY-D2 have previously been shown to be highly immunogenic and for this reason, poorly tumorigenic in the syngeneic host. In contrast MDW40 is highly tumorigenic, poorly immunogenic and completely non-metastatic, differing from the parental tumour line in only the latter phenotype¹⁷. Interaction between tumour cells and the host which do not involve immune stimulation are known to influence metastatic capacity. In particular, metastatic tumour

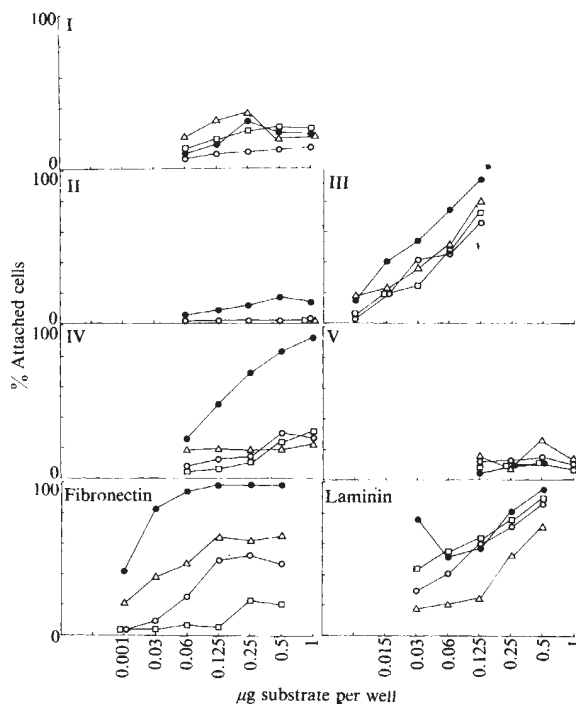


Fig. 1 Adhesion of untreated tumour cells to purified collagens, laminin and fibronectin. Replicate wells in Terasaki Microtest plates (Falcon Plastics) were coated with the following substrates; collagen types I (lathyrus rat skin), II (bovine articular cartilage), III (fetal bovine skin), IV (mouse EHS tumour) and V (human placenta), laminin (mouse EHS sarcoma) and fibronectin (human plasma). The purification of these proteins followed standard procedures²³⁻²⁷. Each well of the Microtest plates was coated with 5 µg bovine serum albumin (BSA) and concentrations of substrate ranging from 0.001 to 1 µg. The BSA and substrates were allowed to dry for 2 h. Tumour cells from tissue culture in log growth phase were washed three times in PBS at 4 °C, adjusted to 5×10^4 per ml in Dulbecco's minimal essential medium (DMEM)/F12 nutrient mixture (3/1, v/v), 15 mM HEPES, pH 7, and 10-µl aliquots of the cell suspensions were added to triplicate wells, incubated at 37 °C for 30 min then turned upside-down and incubated for a further 30 min. Cells adhering to the substrate, and those falling to the base of the droplet were counted and the mean per cent adhering calculated from triplicate counts. Background adhesion to wells coated with BSA alone ranged from 0 to 10%. The data represent the mean of three experiments. ●, MDW40; △, MDAY-D2; ○, MDW40M1; □, MDW40M4.

cells must invade local tissues, move into and out of the circulation and grow at secondary sites, processes which by definition require contact between the tumour cell and host extracellular matrix proteins.

The non-metastatic WGA^R mutant, the parental tumour line and two metastatic revertant lines were compared for their ability to attach to laminin, fibronectin and collagen types I-V using a modification of a sensitive drop assay¹⁸. Compared with the metastatic lines, attachment of MDW40 to collagen type IV and fibronectin was increased (Fig. 1). A small increase in MDW40 attachment to collagen type III was also apparent. Tumour cell attachment to collagens I, II and V was minimal and no differences between the various lines were observed. The four tumour cell lines readily attached to laminin but there were no consistent differences between attachment of the mutant and wild-type cells to this substrate.

The tumour cells were grown in suspension cultures and showed a rounded morphology and negligible attachment to the Falcon tissue culture plastic. With one exception the cells displayed the same rounded morphology following attachment to each of the substrates. MDW40 cells spread only on fibronectin at concentrations >0.03 µg per well and required 2-3 h for optimal flattening. Although MDW40 cells attached to fibronectin at concentrations of <0.03 µg per well they did not flatten, which is consistent with the interpretation that cells require a minimal number of attachment points to spread¹⁹. The weak attachment of the metastatic cell lines to fibronectin

may therefore preclude spreading. MDW40 attached firmly to plastic coated with extracellular matrix produced by endothelial cells in tissue culture. After 3 days of growth on this substrate a tightly packed layer of polygonal shaped cells was formed. The metastatic cell lines remained rounded and loosely attached to plastic coated with endothelial cell extracellular matrix (data not shown). It is known that growth of some tumour cell lines on endothelial cell extracellular matrix results in a more differentiated cell morphology, reflected by firm attachment, cell spreading and formation of non-overlapping cell monolayers³.

In an attempt to mimic the attachment phenotype of the mutant, tumour cells were pretreated with neuraminidase and compared with sham-treated cells for their ability to adhere to laminin, fibronectin and collagen types III and IV (Fig. 2). Neuraminidase treatment had no effect on adhesion of MDW40 cells but greatly enhanced that of MDAY-D2, W40M1 and W40M4 cells to collagen type IV and fibronectin. Similar though less marked effects were observed with collagen type III as substrate, and no differences were observed with laminin. The results indicate that decreased cell-surface sialic acid can increase cell attachment to fibronectin and collagen type IV while not affecting attachment to laminin. Therefore, cell attachment to fibronectin and collagens may be mediated by cell-surface receptors different from those which mediate attachment to laminin. Selective inhibition of hepatocyte attachment to laminin or fibronectin by the same respective proteins in soluble form has led to identical conclusions²⁰.

Our results indicate that a mutation leading to increased cell-surface sialic acid may produce a less adhesive cell. Furthermore, some ricin-resistant mutants of baby hamster kidney cells have been shown to bind less ricin due to masking of the ricin receptors by sialic acid and to be less adhesive to fibronectin-coated plastic than the parental cell line^{6,7}. These results and our own are consistent with the proposal that cell attachment to certain substrates such as fibronectin and collagen type IV

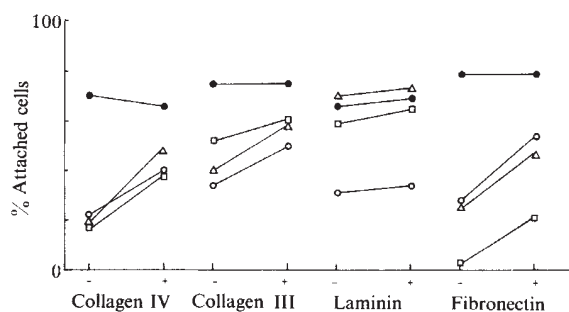


Fig. 2 The effect of neuraminidase treatment on adhesion to collagens, laminin and fibronectin. Cells were washed three times in PBS, and two samples of 10^6 cells in 200 μ l of Dulbecco's PBS, pH 6.9, were incubated with and without 0.02 U of neuraminidase from *Arthrobacter wafoeciens* (Behringwerke) at 37 °C for 30 min. 2 ml of PBS was then added, the cells were centrifuged, the supernatant was removed and a further treatment with 0.02 U neuraminidase carried out. The cells were then washed three times in cold PBS, resuspended at 5×10^4 ml in DMEM/F12 nutrient mixture (3/1, v/v), 15 mM HEPES, pH 7, and kept on ice. The adhesion of treated and untreated cells to fibronectin (0.03 μ g per well), laminin (0.25 μ g per well) collagen III (0.06 μ g per well) and collagen IV (0.25 μ g per well) was measured. The data represent the mean of three experiments. Neuraminidase treatment enhanced binding of the metastatic cell lines to collagen IV ($P < 0.05$), fibronectin ($P < 0.05$) and collagen III ($P < 0.1$), but not to laminin ($P < 0.95$) as determined by a paired *t*-test. ●, MDW40; △, MDAY-D2; ○, MDW40M1; □, MDW40M4.

requires carbohydrate chains which are potential acceptors of terminal sialic acid residues.

The specific glycoconjugates involved in cell attachment have not been clearly defined. Glycolipids terminating in galactose have been shown to mediate homotypic adhesion²¹ and polysialylated gangliosides seem to inhibit fibronectin-mediated cell attachment in a nonspecific manner²². These findings are consistent with our results and with the hypothesis that specific glycoconjugates terminating in galactose (that is, non-sialylated *N*-linked complex or *O*-linked mucin type as well as glycolipids) may be receptors for a lectin-like interaction with fibronectin.

In a manner analogous to ricin binding, sialation of the oligosaccharide may reduce fibronectin binding. These results do not preclude the earlier suggestion that sialic acid increases the net negative charge of the cell and effects a charge repulsion of the cell and substratum²³. However, this is less likely since changes in sialation did not effect cell attachment to laminin.

The avidity of attachment to substrate may change cellular behaviour. Compared with their normal counterpart, transformed cells are known to be less adhesive and less dependent on substratum for growth, properties which may allow the cells to escape from the primary tumour. Firm attachment of cells can induce differentiation² and contact inhibition of growth¹. Our results suggest that the increase in sialation of the metastatic tumour cell surface⁸ can result in decreased attachment to basement membrane proteins collagen type IV and fibronectin, predisposing the tumour cells to increased mobility and decreased growth control by substratum contact.

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Table 1 Comparison of neuraminidase-accessible sialic acid on WGA^R mutant and wild-type cells

Tumour cell lines	Phenotype*	Release of counts from ³ H-N-acetylmannosamine-labelled cells (%)†	Thiobarbituric acid assay (nM sialic acid per mg cell protein)‡
MDAY-D2	Met, WGA ^S , Oua ^S	16.7 ± 5.1	1.91 ± 0.35 (5)
MDW40	Non Met, WGA ^R , Oua ^R	4.1 ± 1.8	0.59 ± 0.13 (4)
W40M1	Met, WGA ^S , Oua ^R	22.2 ± 9.5	1.89 ± 0.06 (2)
W40M4	Met, WGA ^S , Oua ^R	16.0 ± 5.7	1.69 ± 0.12 (3)

* The origin of the highly metastatic (Met) DBA/2 tumour MDAY-D2 has been described in detail previously²⁴. WGA (2–5 μ g ml⁻¹) and ouabain (0.1 mM) added to MDAY-D2 in tissue culture reduced cell growth by 90% (D₁₀) (WGA^S, Oua^S). MDW40 is a ouabain-resistant (Oua^R) mutant (D₁₀ = 3 mM) of MDW4, a non-metastatic WGA^R mutant (D₁₀ = 50 μ g ml⁻¹) of MDAY-D2¹⁴. MDW40 cells have been shown to grow only locally at the site of a subcutaneous inoculation and when 10⁵ cells are injected intravenously less than 5% of the mice die of tumour takes. Recent experiments have demonstrated that 28–35 days after intravenous injection of MDW40 cells, one or a few tumour cells form hybrids with a host cell *in situ*, acquiring the WGA-sensitive phenotype¹⁵. The WGA-sensitive tumour cells selectively dominate the subcutaneous tumour cell population and metastasize to distant organs, resulting in death 60–90 days after tumour cell inoculation. W40M1 and W40M4 are WGA^S, Oua^R resistant tumour cell lines isolated from the liver and spleen respectively of two DBA/2 mice injected subcutaneously with 10⁵ MDW40 cells 90 days earlier.

† Tumour cells, 5×10^5 per ml, were cultured in RPMI plus 10% fetal calf serum and 2 μ Ci ml⁻¹ [6-³H]N-acetylmannosamine. After 48 h, the cells were washed three times in Dulbecco's phosphate-buffered saline (PBS) and samples of 10⁶ cells in 100 μ l of PBS, 5 mM CaCl₂, pH 6.1, were incubated alone (C) or with 0.02 U of neuraminidase (Behringwerke) (N) for 60 min. at 37°C. The cells were washed twice in PBS, solubilized and radioactivity was measured. The formula $(C - N/C) \times 100$ was used to calculate the percentage of neuraminidase-accessible sialic acid¹⁰. The results are the mean ± s.e. of three experiments.

‡ Tumour cells were washed three times and 20×10^6 cells in 0.5 ml PBS, 5 mM CaCl₂, pH 6.1, were incubated with 2 U of neuraminidase (Sigma, type V) for 60 min at 37°C. The cells were pelleted and sialic acid in the supernatant was measured using the thiobarbituric acid assay. Protein was measured using the Bio-Rad reagent and ovalbumin as the standard. The results represent the mean ± s.e. of the number of experiments indicated in parentheses.

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Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour

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The neuroregulation of growth hormone (GH) secretion is mediated in part by a stimulatory, GH-releasing factor (GRF) and an inhibitory peptide, somatostatin¹, both of which reach the adenohypophysis by the hypothalamic-hypophyseal portal system². Somatostatin has been shown to be a tetradecapeptide^{3,4}, a large form of which was later also sequenced⁵. The existence of a GRF is supported by physiological evidence^{6–9} and by reports of GH-releasing activity in hypothalamic extracts^{10–17}. Although several agents with GH-releasing activity have been identified^{14–16}, none is of high potency and fulfils the criteria expected of a physiological GRF^{18,19}. GH-releasing activity observed in extracts of carcinoid and pancreatic islet tumours removed from patients with GH hypersecretion, acromegaly and pituitary adenoma or hyperplasia has been partially characterized^{20–23}. We report here the sequence of a 40 residue GH-releasing peptide purified from a pancreatic islet tumour described earlier by Thorner *et al.*²³. A synthetic replicate of this peptide, termed human pancreatic tumour GRF [hpGRF(1–40)]-OH, co-elutes on HPLC with the native peptide and is highly potent in stimulating GH secretion *in vitro* and *in vivo*. A close structural homology with the peptides of the glucagon-secretin family, and more particularly with PHI²⁴, is recognized. Structure-activity studies show that hpGRF(1–29)-NH₂ has full intrinsic activity and potency *in vitro*.

hpGRF was purified from a pancreatic tumour removed from a subject who presented symptoms and signs of acromegaly and increased GH levels that rapidly abated after surgical removal of the tumour²³. Throughout the purification, we used an *in vitro* method for assaying the ability of a putative GRF to stimulate the secretion of GH by primary cultures of rat pituitary cells (see ref. 25 and Fig. 3 legend).

The tumour was processed in two batches (I and II) of 2.5 and 5 g respectively. Batch I allowed us to optimize extraction and chromatographic conditions that led to the final two-step purification of hpGRF from batch II (see Fig. 1 legend for details). Approximately 6 nmol of >80% pure GRF were obtained, corresponding to an overall recovery of >50%.

Both purification steps yielded one major zone of GRF-like activity. The independent processing of side cuts from the gel permeation or generated during reverse-phase HPLC purification indicated that very closely related molecules having

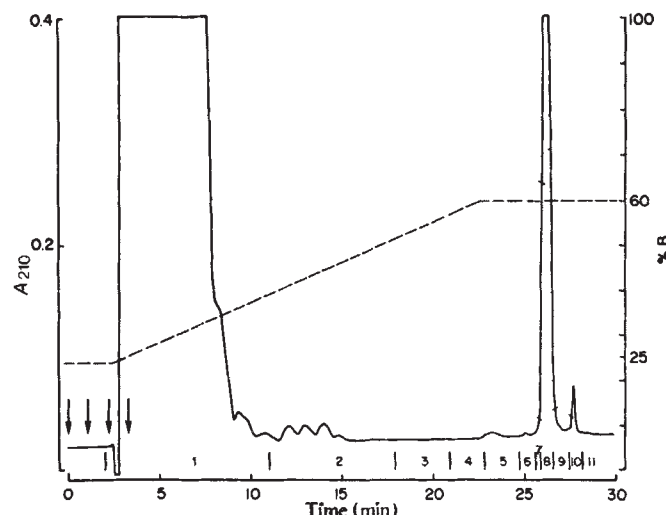


Fig. 1 Extraction and gel permeation chromatography of hpGRF: batch II was lyophilized, polytroned in 10 vols acetone, maintained at -20°C for 2 days and centrifuged. The acetone precipitate was extracted in hot ($>90^{\circ}\text{C}$) acid (1 M acetic acid + 0.1 M HCl) containing 10 mM EDTA and 0.5% β -mercaptoethanol (β -ME) and $5\text{ }\mu\text{g ml}^{-1}$ pepstatin, heated for 2 min, polytroned, chilled and centrifuged. The supernatant was defatted by 3×4 vols ether/petroleum ether (1:2) and applied to a 3.5×120 cm column containing Sephadex G-50 fine and eluted with 3 M acetic acid/0.5% β -ME at a flow rate of 20 ml h^{-1} . Fractions (9.4 ml) were collected, aliquots were lyophilized and assayed in pools. GH-releasing activity was detected in fractions 55–76 corresponding to K_{av} 0.20–0.38. HPLC purification of hpGRF: the zone containing most of the GRF activity (K_{av} 0.24–0.33) was applied through multiple injections (4×1 ml) to a Vydac diphenyl end-capped ($5\text{ }\mu\text{m}$, $300\text{ }\text{\AA}$ pore size, 4.5×250 mm) column (three equivalent runs were performed with the following loads 1/5 and twice 2/5 of total), which was then eluted with an acetonitrile gradient (3–30% in 25 min) in TEAP buffer³⁷. The activity was associated with several absorbance peaks eluting 22–25 min after the start of the gradient (flow rate 1.2 ml min^{-1}). A second HPLC step was performed on the pool of the active fractions from the previous run after partial evaporation of the acetonitrile in a spin vacuum concentrator. A Vydac C_{18} ($5\text{ }\mu\text{m}$, $300\text{ }\text{\AA}$, 4.5×250 mm) column was eluted with an acetonitrile gradient (21–36% in 20 min) at a flow rate of 1.2 ml min^{-1} after multiple injections (2×1 ml) of the concentrated fractions at the relative loads described earlier. All the activity was associated with one symmetrical absorbance peak eluting 21.5 min after the start of the gradient. The total pool of the active fraction (injection volume of 3.5 ml loaded in three aliquots of 1 ml and one of 0.5 ml) was re-run on the same Vydac C_{18} end-capped column at a flow rate of 1.2 ml min^{-1} and room temperature in the gradient conditions shown in the figure ($A=0.1\%$ trifluoroacetic acid (TFA) in water, $B=0.1\%$ TFA in 60% $\text{CH}_3\text{CN}/40\%$ water) to concentrate hpGRF and free it, before sequencing, from β -ME which had been added to each tube at each step in order to retain a high level of biological activity. Indeed, partial oxidation of methionine-27 to methionine sulphoxide leads to a significant loss of activity ($\sim 2\%$ of that of the parent compound) as was shown with synthetic analogues. Fraction 8 was subsequently submitted to composition and sequence analysis. Native hpGRF and hpGRF(1–40)-OH co-elute on HPLC under conditions (Vydac C_{18} , 1.2 ml min^{-1} , 27–31% CH_3CN in 20 min) that would separate them from all other analogues described here including hpGRF(1–40)-NH₂.

GRF activity may be present in the extracts. Their relative amounts, however, could not be more than 5% of the total on the basis of absorbance readings. Furthermore, it is not excluded that these distinct active entities may have been the result of molecular alterations (oxidation of methionine or partial hydrolysis) that occurred during processing of the fractions.

The primary structure of purified hpGRF was determined by Edman degradation in a Beckman 890C spinning cup sequencer, modified according to Wittmann-Liebold²⁶, to be: Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-. The sequencing strategy and results are described elsewhere²⁷ and summarized in Fig. 2 legend.

Fully protected hpGRF(1–40)-NH₂ was synthesized in a step-wise manner on a *p*-methylbenzhydrylamine resin and hpGRF(1–40)-OH was synthesized on a chloromethylated resin

according to the general solid-phase approach of Merrifield²⁸ using experimental procedures described earlier^{29,30}. After complete deprotection and cleavage by hydrofluoric acid, the crude preparations of hpGRF(1-40) and analogues were purified using preparative HPLC techniques developed in our laboratory (see Fig. 3 legend). The abilities of native hpGRF, synthetic hpGRF(1-40)-OH, hpGRF(1-40)-NH₂, and 8-bromo cyclic AMP to stimulate GH secretion by cultured rat pituitary cells are compared in Fig. 3. Both synthetic peptides and the native hpGRF exhibit similar potencies and intrinsic activities in this assay. In multiple culture assays, the minimally effective concentrations of synthetic hpGRF(1-40)-OH are 3-8 pM, the EC₅₀ values 20-100 pM and the maximally effective concentrations are reached by 1 nM. Thus the *in vitro* potency of the synthetic peptide is either similar to or greater than the potencies of the hypophysiotropic peptides isolated from the hypothalamus (thyrotropin-releasing hormone, gonadotropin-releasing hormone, somatostatin¹⁹ and corticotropin-releasing factor³¹).

We have synthesized several extended and shortened analogues of hpGRF(1-40) and tested them *in vitro*. The following peptides exhibited similar (within a factor of 2) potencies to hpGRF(1-40)-OH *in vitro*: hpGRF(1-29)-NH₂, hpGRF(1-32)-NH₂, hpGRF(1-39)-NH₂, hpGRF(1-40)-NH₂, hpGRF(1-40)-Phe-NH₂, hpGRF(1-40)-Phe-OH and hpGRF(1-40)-Phe-Gln-NH₂. These results demonstrate that the N-terminal 29 residues of hpGRF possess all the information required for full *in vitro* activity and suggest that the C-terminal region of hpGRF is not critical for receptor recognition. Full intrinsic activity is observed in hpGRF(1-27)-NH₂ which possesses 10-20% of the potency of hpGRF(1-40)-OH.

hpGRF shows striking structural homology with the peptides of the glucagon family, particularly its new member PHI²⁴ (Fig. 2). hpGRF(1-27) and PHI have 12 common residues in an equivalent position, while 12 additional residues could represent single base changes. We have not observed any GH-releasing activity of PHI, (Gln²⁴)-PHI, vasoactive intestinal peptide or glucagon on anterior pituitary cells. However, the activity of 27- and 29-residue hpGRF analogues suggests that hpGRF may be further processed biologically with maintenance of hypophysiotropic activity.

It is not known whether hpGRF is normally produced in pancreatic islets or if this is an ectopic source for the peptide, or what is the relationship between the putative hypothalamic GRF and hpGRF-(1-40). Natural hpGRF and partially purified

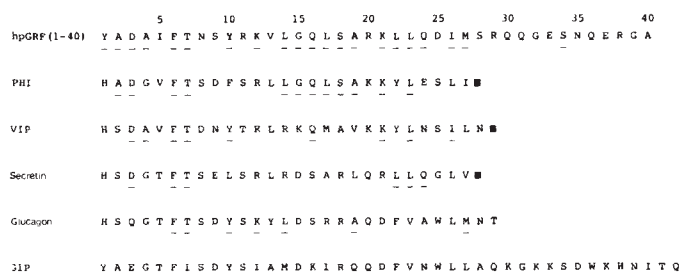


Fig. 2 Comparison of the amino acid sequences of hpGRF and other members of the glucagon-secreting family. Residues of a given structure that are identical with the corresponding one of hpGRF are underlined. The primary structure of hpGRF was determined by Edman degradation. The phenylthiohydantoin derivatives of the amino acids were identified by reverse-phase HPLC³⁸. Several sequencing experiments, each using 0.7-1.2 nmol of peptide, were performed. The peptide was applied directly to the sequencer cup with or without previous treatment with 3-sulphophenylisothiocyanate or after incubation with trypsin, staphylococcal protease or BrCN that cleaved the peptide at different sites. The mixture of fragments was sequenced without purification. The proposed primary structure hpGRF(1-40)-OH shown above satisfies sequence²⁷ and C-terminal (tritiation) analyses⁴⁰, and HPLC data. Amino acid analyses performed using previously reported methodology³⁹ gave the following composition as integers: 5 Asx, 2 Thr, 4 Ser, 7 Glx, 1 Pro, 3 Gly, 4 Ala, 2 Val, 1 Met, 2 Ile, 5 Leu, 2 Tyr, 2 Phe, 3 Lys, 4 Arg. Extra residues are likely to be associated with contaminating peptide(s). Gel filtration and finger printing data²⁷ exclude the possibility that hpGRF is significantly larger. VIP, vasoactive intestinal peptide; GIP, gastric inhibitory polypeptide.

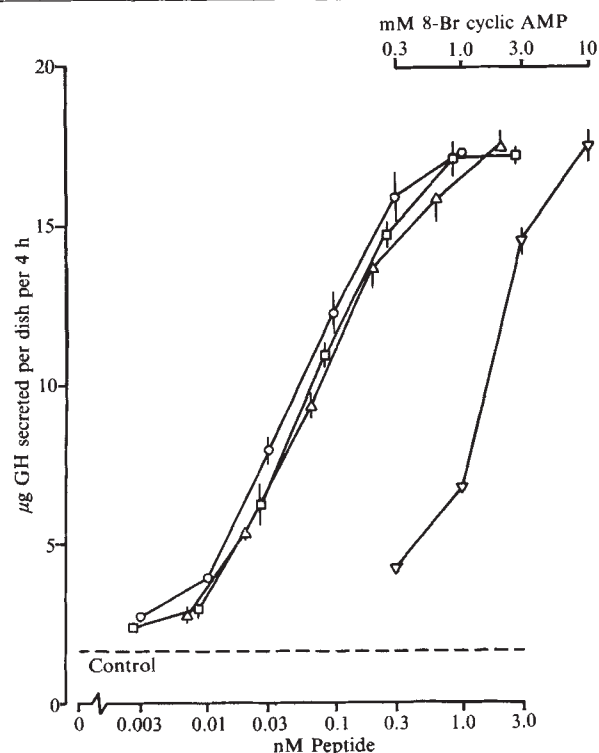


Fig. 3 Effects of native hpGRF (○), synthetic hpGRF(1-40)-NH₂ (□), synthetic hpGRF(1-40)-OH (Δ) and 8-bromo cyclic AMP (▽) on GH secretion over a 4 h period by rat pituitary cells in monolayer culture for 3-5 days. Cells were dissociated and cultured as described in refs 25, 33. Culture medium for this and other assays reported here include 5 nM dexamethasone and 30 pM triiodothyronine. GH levels are estimated by radioimmunoassay using NIADDK National Hormone and Pituitary Program kits prepared by Dr A. Parlow. For purification of the synthetic peptides, cartridges fitting Waters Associates prep LC-500 were packed with 17 μm C₁₈ silica (300 Å; Vydac). A gradient of CH₃CN in TEAP³⁷ was generated by a low-pressure Eldex gradient maker. Desalting of the purified fractions independently checked for purity was achieved using a gradient of CH₃CN in 0.1% TFA. The centre cut was then lyophilized to yield the desired 40-residue peptide, the purity of which can be >98% (J.R., in preparation).

rat hypothalamic GRF, which exhibit similar chromatographic behaviour on Sephadex G-50, can be separated by HPLC. However, other observations suggest that they are structurally similar. There is ample precedence for a close structural relationship between peptides produced ectopically by tumours and those produced normally³². Furthermore, partial sequence analysis of the N-terminal region of highly purified rat hypothalamic GRF showed sequence homology with hpGRF (J.S., J.R. and W.V. unpublished results).

Biologically, hpGRF(1-40) and closely related peptides exhibit many properties expected of a putative GH-releasing factor³³. Thus, synthetic hpGRF and purified rat hypothalamic GRF fractions exhibit parallel dose-response curves and intrinsic activities *in vitro* (Fig. 4). In agreement with our studies¹³ using purified rat hypothalamic GRF, and others^{17,34} using purified hypothalamic or pancreatic tumour GRF, we find that somatostatin non-competitively blocks GH release due to synthetic hpGRF³³. However, even at the highest somatostatin concentrations, hpGRF can still elicit some GH secretion. As with native tumour GRF³⁴, synthetic hpGRF-mediated GH secretion is calcium-dependent³³. In agreement with Cronin *et al.*³⁵ who used native hpGRF, we find that synthetic hpGRF increases intracellular cyclic AMP and that somatostatin attenuates this rise (L. Bilezikjian and W.V., in preparation). The GH-releasing activity of synthetic and native hpGRF is strongly potentiated by pretreatment of cells with glucocorticoids and thyroid hormone³³. hpGRF is also highly potent *in vivo*. A

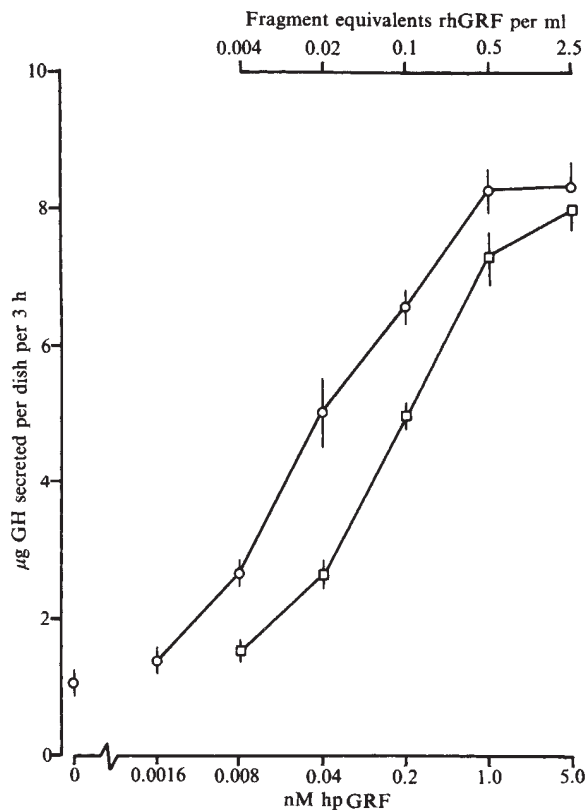


Fig. 4 Effects of synthetic hpGRF (○) and purified rat hypothalamic GRF (rhGRF; □) on secretion of GH by cultured rat pituitary cells. rhGRF was purified as described in ref. 33.

dose-related increase in GH plasma levels was observed (C. Rivier, J.R., J.S. and W.V., in preparation) after administration of from 40 ng per kg to 25 µg per kg of synthetic hpGRF to freely running catheterized rats pretreated with FLA-63, a dopamine β-hydroxylase inhibitor that suppresses spontaneous GH secretion³⁶.

The association of elevated GH levels in the patient with an hpGRF-containing tumour suggests that synthetic hpGRF and its analogues may be powerful GH secretagogues in humans and be of use in the diagnosis and treatment of human disorders.

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Identification of plasmid (pKM101)-coded proteins involved in mutagenesis and UV resistance

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Mutagenesis of *Escherichia coli* by UV and many chemicals is not a passive process and requires the intervention of host functions. The isolation of chromosomal *umuC* mutants, which are nonmutable by many DNA-damaging agents and are slightly sensitive to them, has led to the model that the *umuC* gene product(s) functions in a pathway of 'error-prone repair'¹⁻³. *UmuC* mutants are suppressed by the introduction of the plasmid pKM101⁴; pKM101 was derived from the clinically isolated plasmid R46 by *in vivo* manipulations⁵. The *muc* (mutagenesis, UV and chemical) region of pKM101 is required for this suppression and we have suggested that it is an analogue of the chromosomal *umuC* gene(s)⁶. We have now subcloned the *muc* region onto the high-copy number vector pKB354 and identified two protein products by gel electrophoresis of maxicell preparations. Complementation analysis of *muc* insertion mutations has confirmed the existence of two genes, *mucA* and *mucB*, whose protein products are required for pKM101's effects on mutagenesis and resistance to killing. The molecular weights (MWs) of the *mucA* and *mucB* proteins are 16,000 and 45,000 respectively.

The plasmid pKM101 has played an important part in the Ames test for detecting carcinogens as mutagens^{7,8}. pKM101 confers on its host enhanced capacities for survival and mutagenesis that are *recA*⁺*lexA*⁺ dependent⁹ and apparently inseparable by Tn5 insertion mutagenesis⁶. Such insertions identified an approximately 1.6 kilobase (kb) region of pKM101 necessary for these phenotypes which we termed *muc*.

To determine whether the *muc* region is also sufficient for the expression of these phenotypes, we have now subcloned it into the multiple-copy number vector pKB354 (Ap^R, Tc^R; K. Backman, unpublished). pKB354 is a derivative of pBR322 which has lost the *SalI*/*HincII* site in the *tet* gene, yet remains *tet*⁺. *HpaI* fragments of the pKM101 derivative pGW270 (ref. 10) were subcloned into the *HincII* site in the *bla* gene of

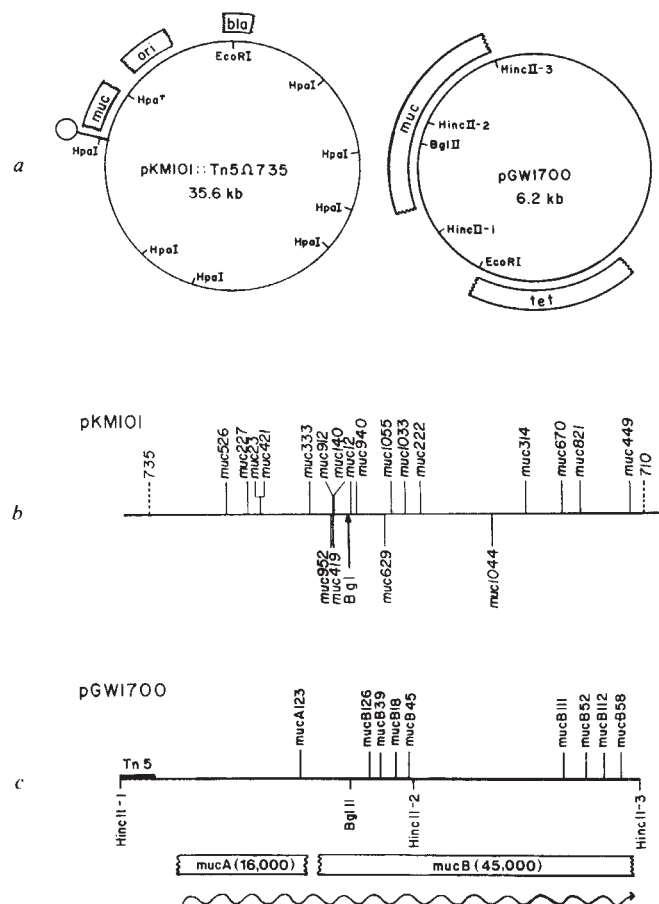


Fig. 1 *a*, Partial restriction map of pKM101::Tn5Ω735 and pGW1700 showing the relative orientation of the *muc* region of the two plasmids. The construction of pGW1700 involved the insertion of a *Hpa*I fragment containing *mucA*, *mucB* and the end of Tn5Ω735 into the *Hinc*II site of the high-copy number vector pKB354. *b*, Expanded map of the pKM101 *muc* region showing the location of Tn5 insertions affecting mutagenesis. pKM101Ω710 and pKM101Ω735 are Tn5 insertions which flank the *muc* region. *c*, Expanded map of the pGW1700 *muc* region showing the location of Tn1000 insertions affecting mutagenesis and the position and direction of transcription of *mucA* and *mucB* (molecular weights in parentheses).

pKB354 and transformed into TK610 (*umuC36*, *uvrA6*¹). Colonies were selected for tetracycline resistance and screened for ampicillin sensitivity and UV mutability. The screen for UV mutability made use of the revertible *his-4* marker of TK610. Tc^RAp^S colonies were spread as patches on minimal media containing a trace amount of histidine (0.02 mM) and irradiated with 2 J m⁻² of UV from a germicidal lamp. Muc⁺ patches were easily identified as they gave rise to as many as 100 His⁺ revertants whereas the control patches rarely had more than one His⁺ revertant.

In this manner, several Muc⁺ derivatives were identified that contained hybrid plasmids in which the *muc* region was inserted into pKB354 in either of the two possible orientations. The expression of *muc* was qualitatively similar regardless of its orientation. One derivative, pGW1700, was selected for further studies. pGW1700 mediates the reactivation of UV-irradiated λ phage (Fig. 3*a*) and suppresses the nonmutability of *umuC36* strains by methyl methanesulphonate (MMS; Fig. 4) to a greater extent than does pKM101. These results indicate that the *muc* region is indeed sufficient for the expression of plasmid-mediated enhancement of survival and mutagenesis.

The proteins encoded by pGW1700 were examined on SDS-polyacrylamide gels using maxicell preparations¹¹ labelled with ³⁵S-methionine. The maxicell procedure takes advantage of the fact that, in *recA* cells, irradiation with appropriate doses of

UV leads to the degradation of chromosomal but not plasmid DNA, thus allowing plasmid-coded proteins to be labelled specifically. pGW1700 encodes two proteins of MWs 16,000 and 45,000 respectively, which are not encoded by the vector (Fig. 2, tracks 1, 2). To determine whether these proteins are products of the *muc* gene(s), we isolated *muc* insertion mutants of pGW1700 generated by the transposable element Tn1000 (ref. 12) and looked for truncated peptides. Initial attempts to obtain pGW1700 *muc*::Tn1000 insertions used GW2100 (*umuC*::Tn5) as the recipient and proved difficult for reasons that are still unknown. Introduction of pGW2, a pkm101 *muc* mutant derived by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine treatment¹³, into GW2100 facilitated the isolation of such mutants. *Muc* insertions isolated in this manner map, by restriction endonuclease analysis, between the *Bgl*II and *Hinc*II-3 sites of pGW1700. Figure 1 compares the sites of the Tn1000 insertions with those of the *muc*::Tn5 insertions previously isolated in pKM101.

Maxicell preparations of the pGW1700 mutants *mucB58*::Tn1000, *mucB112*::Tn1000 and *mucB126*::Tn1000 lack the 45,000 MW protein, which we have identified as the product of the *mucB* gene (Fig. 2, tracks 3–5). Truncated peptides, resulting from translation termination occurring within Tn1000 sequences¹¹, are present in the *mucB58*::Tn1000 and *mucB112*::Tn1000 extracts. Their sizes suggest that the direction of transcription of *mucB* is towards the *Hinc*II-3 site of pGW1700. (The ends of *mucB* are defined by the *mucB58*::Tn1000 insertion and by a fusion of the 5' end of the gene to *lacZ* at the *Bgl*II site; S. J. Elledge and G. C. W., unpublished results.) None of the initial pGW1700 *muc*

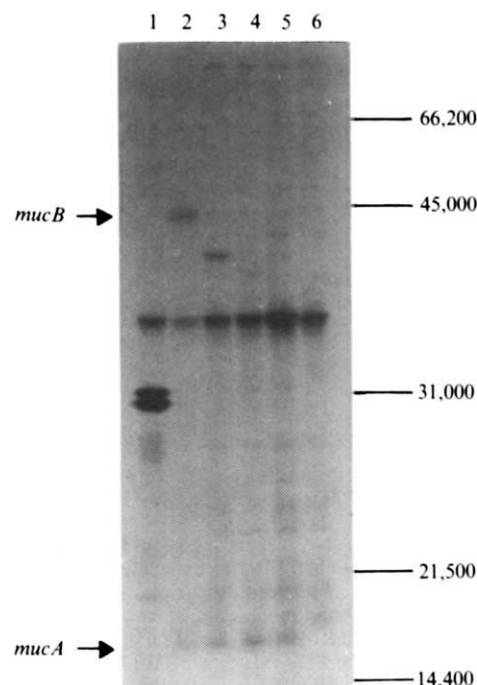


Fig. 2 ³⁵S-methionine-labelled proteins produced in maxicells by the plasmids pKB354 and pGW1700 (*mucA*⁺, *mucB*⁺) and by derivatives of pGW1700 in which Tn1000 is inserted in either the *mucA* gene or the *mucB* gene. Maxicell extracts were prepared from plasmid-containing derivatives of RB901 (*spr*, *recA*) following UV irradiation to 35 J m⁻². Proteins were separated by electrophoresis on a 10–18% gradient polyacrylamide gel and visualized by fluorography. Arrows indicate the positions of the *mucA* and *mucB* proteins. Tracks 1, pKB354; 2, pGW1700; 3, pGW1700 *mucB58*::Tn1000; 4, pGW1700 *mucB112*::Tn1000; 5, pGW1700 *mucB126*::Tn1000; 6, pGW1700 *mucA123*::Tn1000. Molecular weights of the *mucA* and *mucB* proteins were determined by reference to unlabelled protein standards following staining with Coomassie blue. Molecular weights of the standards are indicated on the side.

insertion mutations affected the synthesis of the 16,000-MW protein. We suspected that such mutations were complemented by pGW2 in the GW2100 recipient and therefore went undetected. The use of GW2100(pKM101 *mucA421::Tn5*)⁶ as a recipient permitted the isolation of pGW1700 *mucA123::Tn1000*. *mucA123::Tn1000* mutants fail to synthesize both the 45,000-MW *mucB* protein and the 16,000-MW *mucA* protein in maxicell preparations (Fig. 2, track 6). The apparent polarity of the *mucA123::Tn1000* mutation suggests that *mucA* and *mucB* constitute an operon, the *mucA123::Tn1000* insertion being in the promoter-proximal *mucA* gene.

The compatibility between pKM101 and pGW1700 derivatives has enabled us to carry out complementation analyses of several *muc* alleles which have revealed that both the *mucA* and *mucB* gene products are required for plasmid-mediated mutagenesis and repair. A deletion derivative of pKM101 *mucA421::Tn5* was constructed which lacks the internal *XhoI* fragments¹⁴ of Tn5. Such deletions are reported to relieve polarity effects caused by insertion of the transposon¹⁴. *MucA421::Tn5ΔXhoI* and *mucB112::Tn1000* alleles complement to a moderate extent when tested for the capacity to reactivate UV-irradiated λ phage (Fig. 3b). The original insertion mutation, *mucA421::Tn5*, also complements *mucB112::Tn1000*, but to a lesser degree. Thus it seems that the *mucA421::Tn5* insertion is incompletely polar on *mucB*. Complementation of phage reactivation between *mucB1044::Tn5* and *mucA123::Tn1000* is also observed, but at a very low, yet reproducible, level (Fig. 3a).

Similar results were obtained in complementation analyses of MMS mutability, determined by reversion of the ochre *argE3* marker (Fig. 4). *mucA421::Tn5* and *mucB112::Tn1000* com-

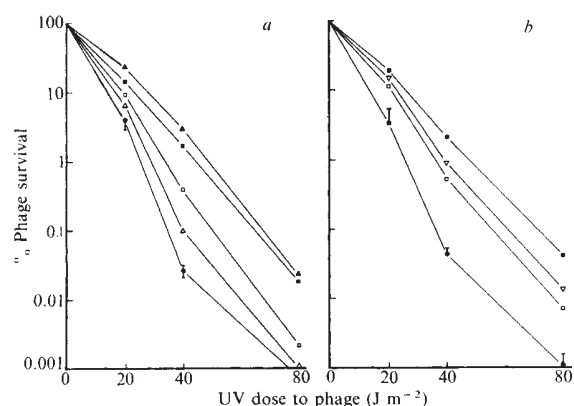


Fig. 3 Reactivation of UV-irradiated λcI857 mediated by pKM101 and pGW1700 and showing complementation between several *mucA* and *mucB* alleles of those plasmids. Reactivation was measured as described previously⁶. a, ●, TK610 (*umuC36*, *uvrA6*); ■, TK610(pKM101); ▲, TK610(pGW1700); □, TK610(pKM101 *mucA421::Tn5*, pGW1700 *mucB112::Tn1000*); △, TK610(pKM101 *mucB1044::Tn5*, pGW1700 *mucA123::Tn1000*). The following strains, also assayed for λcI857 reactivation, gave results similar to those of TK610: TK610(pKM101 *Tn5*), TK610(pGW1700 *mucA123::Tn1000*), TK610(pGW1700 *mucB112::Tn1000*), TK610(pKM101 *mucA421::Tn5*, pGW1700 *mucA123::Tn1000*), TK610(pKM101 *mucB1044::Tn5*, pGW1700 *mucB112::Tn1000*). The range of values for these strains is indicated by the bars. b, ●, TK610(pKM101 *mucA421::Tn5*); ■, TK610(pKM101); ▽, TK610(pKM101 *mucA421::Tn5ΔXhoI*, pGW1700 *mucB112::Tn1000*); □, TK610 (pKM101 *mucA421::Tn5*, pGW1700 *mucB112::Tn1000*). The following strains, also assayed for λcI857 reactivation, gave results similar to those of TK610(pKM101 *mucA421::Tn5*): TK610(pKM101 *mucA421::Tn5ΔXhoI*) and TK610(pGW1700 *mucB112::Tn1000*). The range of values for these strains is indicated by the bars.

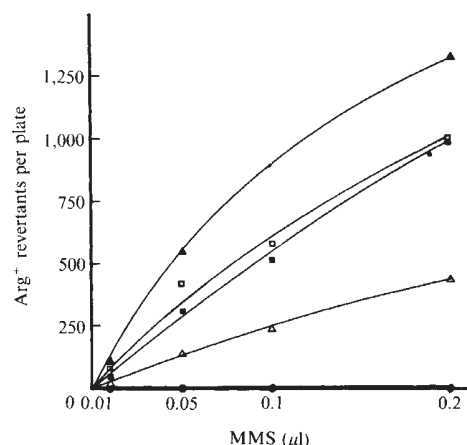


Fig. 4 MMS-induced reversion of the *argE3* mutation mediated by pKM101 and pGW1700 and showing complementation between several *mucA* and *mucB* alleles of those plasmids. To 2 ml of top agar supplemented with limiting (0.05 mM) arginine and nonlimiting concentrations of all other growth requirements were added 0.1 ml of a fresh stationary phase culture and the appropriate amount of a freshly prepared solution of MMS in dimethyl sulphoxide. The top agar was then poured on minimal-glucose plates which were then incubated at 37°C for 4 days. Spontaneous revertants have been subtracted. ●, GW3201(TK610 *argE3*, *zii2::Tn5*); ■, GW3201(pKM101); ▲, GW3201(pGW1700); □, GW3201(pKM101 *mucA421::Tn5*, pGW1700 *mucB112::Tn1000*); △, GW3201(pKM101 *mucB1044::Tn5*, pGW1700 *mucA123::Tn1000*). The following strains, also included in the assay, were indistinguishable from GW3201: GW3201 (pKM101 *mucA421::Tn5*), GW3201(pKM101 *mucB1044::Tn5*), GW3201(pGW1700 *mucA123::Tn1000*), GW3201 (pGW1700 *mucB112::Tn1000*), GW3201(pKM101 *mucA421::Tn5*, pGW1700 *mucA123::Tn1000*) and GW3201(pKM101 *mucB1044::Tn5*, pGW1700 *mucB112::Tn1000*).

plement to mediate MMS mutagenesis to a level equivalent to that mediated by pKM101. Again, the *mucB1044::Tn5* and *mucA123::Tn1000* alleles complement to a lesser degree. We suspect that the differences in the extent of complementation reflect differences in the degree of polarity caused by the *mucA421::Tn5* and *mucA123::Tn1000* insertions. Alternatively, complementation may be affected by gene dosage effects caused by copy number differences between pKM101 and pGW1700 derivatives. No complementation of MMS mutability or phage reactivation was observed between *mucA421::Tn5* and *mucA123::Tn1000* or between *mucB1044::Tn5* and *mucB112::Tn1000*.

These results indicate that both *mucA* and *mucB* gene products are required for mutagenesis and repair, and that the phenotype of *mucA* insertions is not due simply to their polar effects on *mucB*. As we have yet to discern any phenotypic differences between *mucA* and *mucB* mutations, it seems likely that the two gene products act in concert to mediate mutagenesis and repair.

The chromosomal *umuC* locus of *E. coli* has recently been cloned and found to consist of two genes, *umuD* and *umuC*, that code for gene products of MWs 16,000 and 45,000 respectively¹⁵. The pKM101 *mucA* and *mucB* gene products are thus very similar in size to the chromosomally encoded *umuD* and *umuC* gene products, further supporting the hypothesis⁴ that the proteins of the *muc* and *umuC* loci have analogous roles in the processing of damaged DNA that gives rise to mutations. We are now investigating the possibility of complementation between *muc* and *umu* alleles. As no *muc* alleles that we tested complemented the *umuC36* mutation carried by the host, either the individual *muc* gene products are insufficiently homologous to complement separately the corresponding *umu* gene products or the *umuC36* mutant is defective in both *umuD* and *umuC*. The identification of the *mucA* and *mucB* proteins

should facilitate a systematic analysis of the biochemistry of error-prone repair.

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A transposon in *Streptococcus faecalis* with fertility properties

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Certain strains of streptococci have been shown to be capable of conjugatively transferring drug resistance determinants in the apparent absence of plasmid DNA^{1–6}. The transferrable tetracycline resistance (*Tc*^r) marker of *Streptococcus faecalis* DS16 has been located on a transposon, designated *Tn 916*^{2,4,7}. We show here that when chromosomal *Tn 916* is conjugatively transferred it inserts into a number of different sites on the recipient chromosome. Our studies also imply that transfer and transposition share a common step, which may be excision of the transposon from the donor chromosome.

The ability of *Tn916* to transfer from plasmid-free donors (see Table 1 for description of bacterial strains) in overnight filter matings is shown in the data of Table 2. DS16C3 is able to transfer *Tc*^r at a frequency of about 10⁻⁸ to the plasmid-free recipient strain JH2-2. Transconjugants such as CG130 are capable of transferring *Tc*^r to the isogenic recipient JH2SS at a similar frequency. To investigate the nature of this transfer event, chromosomal DNA isolated from the donor and transconjugant strains listed in Table 2 was digested with *Hind*III restriction endonuclease, resolved by agarose gel electrophoresis, and transferred to nitrocellulose paper using the Southern technique⁸. The transferred DNA was probed with a ³²P-labelled *Eco*R1 restriction fragment (F') of pAM211 (a derivative of pAD1, a resident plasmid of DS16) that contains the entire transposon. There are no *Eco*R1 sites within *Tn916* and about 85% of the F' fragment consists of *Tn916* sequences. As *Tn916* contains a single *Hind*III site, two chromosome-transposon junction fragments should be resolvable after hybridization with the probe. The resulting autoradiogram (Fig. 1a) shows that three different transconjugants obtained from the DS16C3×JH2-2 mating, detailed in Table 2, display hybridization patterns (lanes 2–4) that differ from those seen in the donor (lane 1) as well as from each other. One such

transconjugant, CG140, gave rise to two chromosome-transposon junction fragments (X and Y), whereas two other transconjugants, CG110 and CG130, gave rise to four or more bands. Figure 1a also shows the hybridization profiles of transconjugants derived from the secondary mating described in Table 2 that had used strain CG130 as the donor. Again the hybridization bands of the transconjugants (lanes 5–7) differed from those of the donor as well as from each other. Strain CG132 gave rise to a multiple band pattern, whereas two other transconjugant strains, CG131 and CG133, exhibited the simpler banding pattern.

The variation in hybridization profiles amongst different transconjugants implies that *Tn916* inserts at different sites on the recipient chromosome. These findings also argue against the possibility that *Tn916* existed on a plasmid which had escaped physical detection. If the latter had been the case, identical patterns should have been observed in the transconjugants.

The patterns of multiple bands observed with transconjugants CG110, CG130, and CG132 were reproducible, and do not represent incomplete digestion products. An autoradiogram of a Southern blot hybridization experiment involving *Eco*R1 digested chromosomal DNA and ³²P-labelled *Tn916* probe is shown in Fig. 1b. As expected, a single hybridizing fragment was detected in the *Eco*R1 digested chromosomal DNA from strains CG133, CG131, and DS16C3 (strains that gave rise to two hybridizing fragments in the *Hind*III digestion experiments—see Fig. 1a). By contrast, those transconjugants that gave rise to complex band patterns following *Hind*III digestion (CG132, CG130, and CG110) gave rise to more than one hybridizing fragment when digested with *Eco*R1. In every case, the hybridizing fragments following *Eco*R1 digestion of chromosomal DNA were larger than ~15 kilobase. These data strongly suggest the presence of non-tandem multiple copies of the entire transposon in strains CG110, CG130, and CG132; however, the possibility that major rearrangements of the transposon give rise to these data cannot be completely eliminated. We note that restriction enzyme analyses of several pAD1::*Tn916* plasmids formed by transposition from the CG110 chromosome revealed a typical ~15 kilobase insertion (data not shown).

Tc^r transconjugants of JH2-2 that exhibit altered transfer frequencies in secondary matings were readily obtainable, and usually represented about 20% of the original transconjugants. Strain CG110 was capable of donating *Tn916* at frequencies of about 10⁻⁶ (Table 2). To investigate the transposition of *Tn916* in such a strain the conjugative haemolysin plasmid pAD1 was introduced into strains CG110 and CG130, and the frequency of *Tn916* transposition from the chromosome to pAD1 was measured by determining the number of *Tc*^r, hyperhaemolytic transconjugants obtained after filter-mating of these

Table 1 *S. faecalis* strains and relevant properties

Strain	Chromosomal genotype	Plasmid content	Reference
DS16C3	<i>tet</i>	None	4
JH2-2	<i>rif fus</i>	None	21
JH2SS	<i>str spc</i>	None	22
CG110	<i>rif fus tet</i>	None	This paper
CG130	<i>rif fus tet</i>	None	This paper
OG1-RF (pAM211)	<i>rif fus</i>	pAM211(<i>Tn916</i> inserted into <i>Eco</i> R1 F fragment of pAD1)	4
CG180	<i>rif fus</i>	pAM180 (pAM81:: <i>Tn916</i>)	This paper
CG190	<i>str spc</i>	pAM190(<i>Tn917</i> inserted into <i>Eco</i> R1 B fragment and <i>Tn916</i> into <i>Eco</i> R1 D fragment of pAD1)	This paper

Table 2 Tn916 transfer from plasmid-free donors

Donor strain	Recipient strain	Frequency of Tc^r transconjugants per recipients*	Representative transconjugants
DS16C3	JH2-2	1×10^{-8}	CG110 CG130 CG140
CG130	JH2SS	2×10^{-8}	CG131 CG132 CG133
CG110	JH2SS	4×10^{-6}	

* Filter matings were carried out as described in ref. 4, using Difco antibiotic medium no. 3 (AB3), and an initial ratio of one donor per ten recipients. Antibiotics were used in selective agar plates at the following concentrations: tetracycline, $10 \mu\text{g ml}^{-1}$; rifampin, $25 \mu\text{g ml}^{-1}$; fusidic acid, $25 \mu\text{g ml}^{-1}$; and streptomycin, $1000 \mu\text{g ml}^{-1}$. For reasons discussed in ref. 4, we generally express frequencies in terms of recipients (rather than donors) in cases where the values are very low. Frequency of spontaneous Tc^r mutations was less than 10^{-10} .

donor strains with JH2SS. The hyperhaemolytic phenotype results from an insertion within or near the haemolysin determinant of pAD1, and can be readily detected by an increased diameter of haemolysis zones on horse blood agar plates⁴. The frequency of hyperhaemolytic, Tc^r transconjugants was found to be more than 200 times higher when CG110 was the donor strain (4.9×10^{-6} per recipient) than when CG130 was the donor strain (1.8×10^{-8}). pAD1, *per se*, transferred equally well ($\sim 10^{-2}$) from both hosts. Similar results were obtained with another independently isolated variant, CG100, which transferred Tn916 at frequencies about 10 times higher than normal; transposition from the CG100 chromosome to pAD1 was similarly increased. These data indicate Tn916 transfer and transposition may share a common step.

When located on a conjugative plasmid, Tn916 was found to excise at relatively high frequency upon transfer to a recipient strain. pAM180 is a derivative of the ~ 26 kilobase erythromycin-resistance (Em^r) plasmid pAM81 with Tn916 inserted into the *EcoR*I fragment G. When donor strains containing pAM180 were filter-mated with JH2SS, a high degree of segregation of the Em^r and Tc^r determinants occurred, as can be seen in the data of Table 3. Although both the Em^r and Tc^r determinants transferred at similar frequencies, a significant proportion of erythromycin selected transconjugants (43–77%) were not resistant to tetracycline.

Of those transconjugants selected for tetracycline resistance, however, greater than 90% were also erythromycin resistant.

Interestingly, in some of the erythromycin selected transconjugants that were tetracycline resistant Em^r and Tc^r were no longer linked, as implied by a great reduction in the transfer frequency of Tc^r , but not Em^r , in secondary matings. The loss of the Tc^r determinant from the plasmid possibly relates to a zygotic induction of one or more Tn916-related recombination enzymes.

To determine the structural integrity of pAM81 DNA sequences in the various types of transconjugant, DNA from representative plasmids was digested with *Hind*III and analysed by agarose gel electrophoresis (Fig. 2a). Plasmid DNA isolated from two $Em^r Tc^r$ transconjugants in which Em^r and Tc^r were no longer linked (CG184 and CG186) exhibit a restriction pattern identical to that seen for pAM81. (Note the two fragments of pAM180 that had contained Tn916 sequences were lost, and pAM81 fragment G reappeared). It is presumed that Tn916 transposed to the bacterial chromosome in these strains since they were tetracycline resistant and capable of transferring Tn916 at very low frequency. Plasmid DNA from strain CG187 ($Em^r Tc^r$) also gave rise to a restriction pattern identical to that of pAM81. In contrast, plasmid DNA from strain CG181 ($Em^r Tc^r$), in which both resistance determinants remained linked, displayed a restriction pattern identical to that of the pAM180 donor. These data support the view that transposition involves an excision followed by insertion, but that insertion is not necessarily coupled to excision—in which case the excised element is lost.

Analogous results were obtained in matings that used as a donor strain CG190. This strain harbors a pAD1 derivative (pAM190) with two transposon insertions: Tn917 (Em^r) and Tn916 (see Table 1). The Tn916 insertion gave rise to a nonhaemolytic phenotype, yet the strain displays a hyperhaemolytic phenotype on blood plates that contain tetracycline ($10 \mu\text{g ml}^{-1}$). This drug-influenced phenotype has been observed previously in certain pAD1::Tn916 derivatives containing transposon insertions into or near the haemolysin determinant⁹. CG190 transferred Em^r at a frequency of $\sim 2 \times 10^{-4}$ per donor to JH2-2 in overnight broth matings; and Tc^r derivatives (for example CG191) as well as Tc^r derivatives in which the Em^r and Tc^r determinants were no longer linked (CG194) were found among the transconjugants at a frequency of about 4%. Agarose gel electrophoresis of *EcoR*I digested plasmid DNA from such transconjugants (Fig. 2b) revealed the presence of a fragment (*EcoR*I D fragment) that had been missing in the original pAM190 DNA as a result of Tn916 insertion. Most significant was the additional observation that transconjugants CG191, CG192, and CG194 all regained the haemolytic phenotype, implying that the excision of Tn916 was precise.

Fig. 1 Autoradiograms obtained from filter-blot hybridization analyses of chromosomal DNA from Tc^r transconjugants. *a*, *Hind*III digested DNA ($1-2 \mu\text{g}$) from transconjugants CG110, CG140, and CG130, (lanes 2–4 respectively) which were obtained using DS16C3 (lane 1) as donor. Lanes 5–7 contain *Hind*III digested DNA from transconjugants CG131, CG132, and CG133, respectively, which were obtained using CG130 as donor. Fragments marked x and y denote presumed chromosome-transposon junction fragments. Lane 8 contains *Hind*III digested pAM211 DNA and hybridizing bands are approximately 18.5 Kb and 7.5 kb. *b*, *EcoR*I-digested DNA ($1-2 \mu\text{g}$) from strains CG133, CG132, CG131, CG130, CG110, and DS16C3, (lanes 1–6 respectively). Lane 7 contains *EcoR*I digested pAM211 DNA from which the probe had been made. Isolation of DNA by buoyant density gradient centrifugation and agarose gel electrophoresis was as described in ref. 4. DNA transfer and hybridization were essentially according to Southern⁸ as modified by Wahl *et al.*²³, and used a BRL blot transfer system. The *EcoR*I F' fragment of pAM211 was purified by electroeluting the DNA from an agarose gel slice placed in a dialysis bag filled with electrophoresis buffer that had been embedded in 1% agarose. Following electrophoresis⁴ the DNA was recovered by ethanol precipitation. Nick translation of pAM211 *EcoR*I F' probe DNA with either ^{32}P -dCTP (670 Ci mmol^{-1}) or ^{32}P -dATP (410 Ci mmol^{-1}) was carried out using a nick-translation kit.

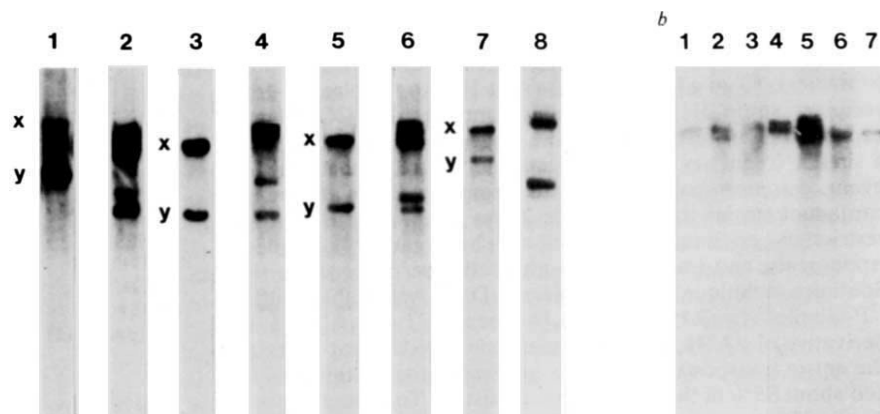
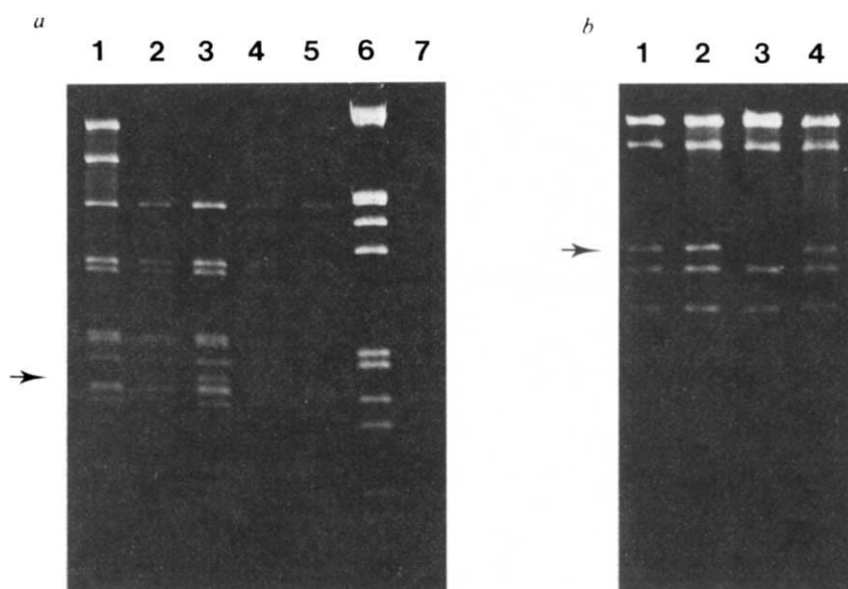


Fig. 2 Excision of Tn916 from plasmid DNA after transfer to recipients. *a*, HindIII digests of transconjugant plasmid DNAs derived from the CG180 donor. Lanes 1 and 2 contain plasmid DNA from CG180 and CG130 (pAM81), respectively. The arrow marks fragment G, no longer present after Tn916 insertion into pAM81. Lanes 3–5 contain plasmid DNA from the *Em^rTc^r* transconjugants CG184 and CG186, and the *Em^rTc^s* transconjugant CG187. Lane 7 contains plasmid DNA from the *Em^rTc^r* CG181 strain, and lane 6 contains λ DNA digested with *Eco*R1 and *Hind*III. *b*, *Eco*R1 digests of transconjugant plasmid DNAs derived from the CG190 donor. Lanes 1–4 contain plasmid DNA from the *Em^rTc^s* transconjugants CG191 and CG192, and the *Em^rTc^r* transconjugants CG193 and CG194. The restriction pattern obtained for pAM190 is identical to that of CG193 (data not shown). The arrow marks the *Eco*R1 fragment D of pAD1, lost as a result of Tn916 insertion and regenerated after excision. The highest molecular weight band in lanes 1, 2 and 4 represents a doublet and includes the pAD1 *Eco*R1 A and B::Tn917 fragments; whereas the highest molecular weight material in lane 3 represents a triplet which includes the *Eco*R1 D::Tn916 fragment. (Broth matings were performed as in ref. 24.) Agarose gel electrophoresis utilized a 1% (Fig. 2a) or 0.7% (Fig. 2b) gel and Tris-borate buffer²⁵.



Transconjugants in which the *Em^r* and *Tc^r* markers remained linked, such as CG193, displayed an *Eco*R1 restriction pattern like the donor and were, as expected, nonhaemolytic.

On the basis of information known thus far we suggest the following model for Tn916 behaviour. When located on the bacterial chromosome Tn916 may spontaneously excise at low frequency. Although there is no direct evidence for the form the excised element might take, we think that it is most probably a circular molecule incapable of vegetative replication. Assuming that induction of key enzymes accompanies the excision step, the element now has the potential to: (1) insert back into the chromosome at a new, or perhaps the original site; (2) insert into a resident plasmid; (3) transfer into a recipient strain (if conditions warrant it); or (4) segregate. Conjugation could be viewed as occurring by a plasmid-like process, with zygotic induction of appropriate enzymes facilitating insertion into the recipient chromosome. The generation of multiple copies of Tn916 in some transconjugants could arise by secondary transpositions (still via excision-insertion) from a replicated portion of the chromosome to an unreplicated region. Completion of

replication thus results in one of the daughter chromosomes having two transposons. Conceivably when Tn916 enters a new cell, a certain amount of time (a generation or so?) may be required to achieve levels of 'repressor' sufficient for stable maintenance of the element at one site. During this time there could be a tendency to move from one site to another.

In view of the increasing reports of 'plasmid-free' transfer of resistance determinants, especially among the streptococci, Tn916 may represent a prototype of a new class of genetic elements that could be referred to as 'conjugative transposons'. In this regard Smith and Guild¹⁰ have recently obtained evidence suggesting that the conjugative multiple resistance element originating in *S. agalactiae* B109 may be located on a large transposon.

It is important to note that homology has recently been demonstrated between Tn916 and the transferable *Tc^r* determinants (classified as *tetM*) of *S. pneumoniae* and *S. agalactiae*^{11,12} as well as with a chromosomal *Tc^r* determinant (not yet shown to be transferable) in *S. mutans*¹³. Recent reports of transferable nonplasmid elements in *Clostridium difficile*¹⁴ and *Bacteroides fragilis*^{15–18} suggest that such elements may exist in a variety of prokaryotic genera (including Gram negative bacteria). Although certain R-plasmids in *Escherichia coli* (IncJ)¹⁹ and *Haemophilis*²⁰ prefer to be integrated in the chromosome, their possession of replication functions and dependence on host recombination functions to integrate distinguishes these elements from Tn916.

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Table 3 Segregation of *Em^r* and *Tc^r* in filter matings involving CG180 donors

Experiment	<i>Em^r Tc^r</i> transconjugants		
	Erythromycin selected*	Tetracycline selected	Erythromycin selected <i>Em^r Tc^r</i> transconjugants with unlinked markers
1	27/47 (57%)	41/43 (95%)	6/12
2	16/48 (33%)	46/48 (96%)	1/12
3	11/48 (23%)	44/48 (92%)	1/10

Tn916 was inserted into pAM81 to generate pAM180 via overnight filter matings between CG110 (pAM81) and JH2SS followed by selection for tetracycline resistant transconjugants capable of transferring *Tc^r* linked to *Em^r* in secondary matings. (The plasmid pAM81, originally identified in *S. faecalis* DU81 is capable of conjugal transfer in filter matings at frequencies of about 10^{-4} per donor, Y. Yagi and L. Dempsey, personal communication.) Each donor was derived from a single colony isolate and was filter mated with JH2SS (JH2-2 in secondary matings) as described in Table 2. Frequency of *Em^r* and *Tc^r* transconjugants was $0.6\text{--}1.5 \times 10^{-5}$ per donor.

* Although *Em^rTc^s* transconjugants appeared readily, random analyses of the CG180 donor failed to detect any *Tc^s* colonies (135/135 were *Em^rTc^r*) implying dissociation of *Tc^r* occurred after, or during, transfer.

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Regulatory sites for *his3* gene expression in yeast

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The expression levels of many genes are regulated in response to particular environmental or developmental cues. A simple regulated gene can conceptually be divided into three elements: structural components that encode the gene product, promoter elements that are essential for gene expression, and regulatory elements responsible for changing the level of expression after a specific stimulus^{1,2}. When cells of the yeast *Saccharomyces cerevisiae* are subjected to amino acid starvation, *his3* and many other amino acid biosynthetic genes are expressed threefold above the basal level^{3,4}. Previously, I isolated mutations mapping outside the structural gene that severely reduce or eliminate *his3* expression^{5,6}. These mutations define two distinct *his3* promoter elements located 115–155 and 32–52 base pairs (bp) from the site of transcriptional initiation. Here I describe 18 small deletion mutations, some of which express *his3* at the basal level but are unable to increase the level of expression in the appropriate physiological conditions. These define two regulatory sites located 32–41 and 80–100 bp upstream from the site of transcriptional initiation, and they strongly suggest that these regions are necessary for the positive regulation of *his3* expression. I consider the results in terms of models for the general control of amino acid biosynthesis.

his3 encodes imidazoleglycerol phosphate dehydratase (IGPD), an enzyme involved in histidine biosynthesis. Its expression is regulated coordinately with the genes involved in amino acid biosynthesis. When yeast cells are starved of histidine, methionine or tryptophan by adding appropriate inhibitors to the growth medium, the levels of IGPD increase about threefold (Table 1). In addition, *his3* expression is controlled in the expected manner by the *aas2* and *tra3* genes.

It is probable that *his3* is regulated solely as a function of general amino acid biosynthesis: first, IGPD levels do not depend on the presence or absence of histidine in the growth medium (Table 1). Second, growth conditions resulting in the selective induction of histidine biosynthetic enzymes have never been found. Third, mutations selected for defective regulation of histidine enzymes always affect other amino acid biosynthetic enzymes also³. These experiments, which fail to demonstrate histidine-specific regulation in yeast, are identical to those that clearly establish such control in *Salmonella typhimurium*.

The mapping positions of 18 mutations of the cloned *his3* gene are presented in Table 2. Each mutation deletes sequences (ranging between 9 and 66 bp) within the untranscribed region located 6–138 nucleotides upstream from the 5' end of the gene. Using standard techniques, the mutant genes were introduced back into yeast cells so that the resulting strains contained one copy of the cloned DNA per cell at the normal *his3* chromosomal location (for experimental details see Fig. 1 legend). Strains harbouring these cloned mutant alleles were

always grown in medium containing histidine so that *his3* expression was gratuitous for cell growth; inducing conditions were achieved by starving the cells of tryptophan. The IGPD levels are shown in Table 2. Two strains were examined for each *his3* allele. One strain contained all the original transforming DNA sequences; the other lacked the vector sequences and is equivalent to replacement of the original *his3*-532 mutation by the allele of interest (see Fig. 1 legend). The IGPD levels for both strains of any given mutant were always the same. Of the 18 mutations tested, 9 regulate *his3* expression properly. However, strains with any of the nine mutations produce IGP constitutively, that is, the enzyme levels are the same in normal conditions and during amino acid starvation. Thus, these nine mutations have the expected properties of *his3* regulatory mutations.

his3 regulatory sites are defined by comparing the DNA sequences of mutations that either regulate or fail to regulate *his3* expression. The simplest interpretation of the data assumes that: (1) a subset of the sequences deleted by a regulatory mutation is essential for normal regulation; and (2) sequences deleted by correctly regulated derivatives are unimportant. Such analysis, when applied to all the derivatives, leads to the simple and internally consistent description of *his3* regulatory elements illustrated in Fig. 2 and discussed below. It is difficult to disprove alternative explanations for the phenotype of any given mutation, and more complex models can be advanced to explain the data.

The extent of *his3*-Δ22 indicates a regulatory site that includes a sequence between 32 and 41 bp upstream from the start of transcription (nucleotides –32 to –41). Deletion of this region accounts for the regulatory defects of five other mutations (*his3*-Δ20, Δ21, Δ23, Δ24 and Δ25). As yet, the DNA sequences sufficient to constitute this regulatory site are not well mapped. However, sequences further upstream than –52

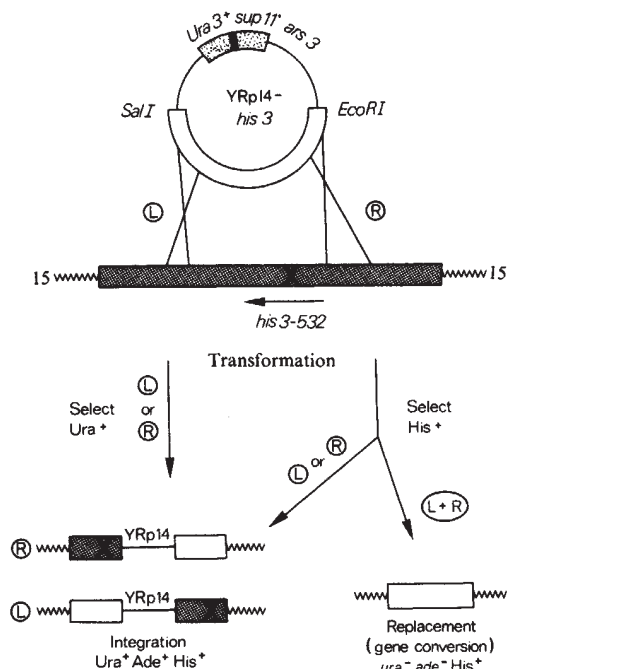
Table 1 Regulation of the *his3* gene

Strain	Genotype	Complete	–His	–Trp	+AT	+Eth
KY29	<i>trp1-289 his3⁺</i>	1.0	1.0	3.0	3.0	3.2
KY137	<i>trp1-289 his3-532</i>	<0.1	NG	<0.1	NG	NT
KY107	<i>aas2-5039 his3⁺</i>	1.0	1.1	NT	1.5	1.3
KY108	<i>tra3-1 his3⁺</i>	3.0	3.0	NT	3.5	3.4

The yeast strains used were: KY29 (*mat-a ura3-52 trp1-289*) isolated by M. Thomas as strain M1-2B; KY137 (*mat-a ade2-1 ura3-1 ura3-2 trp1-289 his3-532 can1⁺*) isolated by S. Scherer; KY107 (*mat-a aas2-5039*) and KY108 (*mat-a tra3-1*) both obtained from G. Fink³. Because *tra3-1* renders strains temperature-sensitive for growth, all cultures were incubated at 27°C with shaking. Complete medium contained 2% glucose, 0.67% yeast nitrogen base without amino acids (Difco), and 0.1 mg ml^{–1} each of histidine, tryptophan, uracil and adenine. In these conditions, the doubling time was 2 h for all strains except KY108, which doubled every 3 h. Strains incubated in complete medium lacking histidine (–His) grew at the same rate, except for KY137 which did not grow (NG). Cells grown in either of these non-starvation conditions were collected in the middle of exponential growth ($A_{600} = 2$ or $\sim 2 \times 10^7$ cells ml^{–1}). To achieve amino acid starvation, cells were first grown in complete medium until A_{600} was ~ 1.0 , concentrated by centrifugation, washed once with distilled water, and then resuspended in complete medium that either lacked tryptophan (–Trp), lacked histidine but contained 10 mM aminotriazole (+AT), or contained 20 μM ethionine (+Eth). The resuspended cells were incubated for a further 6–9 h; the cells undergo an average of one more cell division before their growth is arrested as a result of Trp, His or Met starvation. To determine the levels of IGPD activity, cells were collected, washed twice with water, permeabilized with 1.5% chloroform at 37°C for 20 min, pelleted by centrifugation, and resuspended in 0.1 M triethanolamine, pH 7.7. The enzyme assay was performed as described previously¹² with minor modifications (to be published elsewhere). The enzyme levels are normalized to both the number of cells assayed and to the amount of absorbance at 290 nm released after chloroform treatment. The activity levels shown are relative to those of wild-type yeast grown in complete medium (defined as 1.0). For enzyme levels around 1.0, the error is $\sim 10\%$.

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Fig. 1 Determining the phenotypes of cloned *his3* derivatives. The wild-type *his3* DNA used in these experiments (Sc2605) is a 6.1-kilobase pair (kb) fragment generated by *EcoRI* and *SalI* cleavage¹². In addition to the entire *his3* locus, it contains 2.9 kb flanking the 3' end and 2.5 kb flanking the 5' end. The isolation, characterization and DNA sequence determination of the mutant derivatives of Sc2605 will be described elsewhere. The upper part of the figure shows the structure of *his3* hybrid molecules. All *his3*-containing DNA fragments (open boxes) were cloned into YRp14 (solid line with dotted boxes; see ref. 16), a vector containing the *ura3* gene, the ochre suppressing version of a tyrosine-inserting tRNA (*SUP11*), and *ars3*, a sequence that inefficiently causes hybrid molecules to replicate autonomously. YRp14 hybrid DNAs were introduced into KY137, a strain containing the ochre suppressible allele *ade2-1*, nonrevertible alleles *ura3-1,2* and *his3-532*, and the revertible *trp1-289* allele. Chromosome 15 of this strain is indicated by a wavy line below the YRp14-*his3* hybrid molecule. The shaded box indicates the 6.1-kb region homologous to the cloned *his3* derivatives; X marks the location of the *his3-532* mutation¹³. *Ura*⁺ or *His*⁺ transformants were selected after 5 days of growth at 30 °C. All cloned *his3* fragments produced an equal number of *His*⁺ and *Ura*⁺ transformants for a given amount of DNA, except for Sc2884 (*his3-Δ38*) which produced no *His*⁺ colonies. Essentially all such transformants were mitotically stable for the marker selected in the initial transformation event, indicating that the transforming sequences recombined with genomic DNA. Transformants containing autonomously replicating hybrid molecules were obtained only after ≥10 days. This is probably due to the inefficiency of *ars3*¹⁶ and to lethality associated with multiple copies per cell of tyrosine-inserting suppressor tRNAs¹⁴. The figure shows recombination with the *his3* locus of chromosome 15. The L cross-over occurs to the left of the *his3-532*, while the R cross-over occurs to the right of this allele. The resulting structures are shown at the bottom. The YRp14 vector sequences which include *ura3*⁺ *sup11*⁺ *ars3* are indicated by a solid line. Almost all the *Ura*⁺ transformants were also *His*⁺ *Ade*⁺ while the *His*⁺ transformants fell into two classes. The class of *His*⁺ transformant that was also *Ura*⁺ *Ade*⁺ is genotypically equivalent to the *Ura*⁺ transformants. In this case, the entire YRp14 hybrid molecule has integrated into the genome. As most of the homology between the transforming DNA and the genome is *his3* DNA, integration probably occurs at the *his3* locus; this was true for all cases tested explicitly. The transformants that arise by integration are due to a single L or R cross-over. The other class of *His*⁺ transformant is *ade*⁺ *ura*⁺ and results from replacement of the *his3-532* allele by the transforming allele, probably via gene conversion¹⁵. This class is equivalent to the double (L+R) cross-over. Both classes of transformants should have one copy of the transforming DNA per cell. For the integration class, multiple copies of the *SUP11*-containing transforming DNA per haploid genome should be lethal and for the replacement class, it is difficult to envisage mechanisms that could result in multiple copies of *his3* and none of *ura3*. Indeed, hybridization analysis supports the above contentions. It is possible that cross-overs occur between the *his3-532* and the cloned *his3* alleles—this would generate either the wild-type allele and/or the double mutant. However, as these alleles are very close (less than 250 bp; see ref. 13) recombination between them is rare⁵. In this regard, note that the phenotype of each allele was tested in two independent transformants.



or downstream from -32 are unlikely to be involved because both *his3-Δ26* (which deletes nucleotides -53 to -80) and *his3-Δ18* (nucleotides -32 to -21 deleted) are regulated properly.

The structures of the remaining deletion mutations suggest a second regulatory site: *his3-Δ28* (nucleotides -106 to -53 deleted) and *his3-Δ29* (nucleotides -106 to -73 deleted) fail to regulate *his3* expression. However, the related pair that differ only in the upstream breakpoint (*his3-Δ26* lacking -80 to -53 and *his3-Δ27* lacking -80 to -73) are regulated properly. By subtraction, these results strongly suggest a second regulatory site that includes at least a subset of the sequences between -80 and -106. The upstream boundary of this site is defined further by deletions 32-35. These mutations remove sequences between -101 and -137, but do not prevent *his3* regulation. Some of them do seem, however, to have reduced basal levels of expression.

Thus, there seem to be at least two regulatory elements for *his3* expression separated by at least 30 bp. The downstream element maps between -32 and -52 and includes a region localized between -32 and -41, the upstream element maps somewhere between -80 and -100.

It is worth comparing the sequence of the *his3* regulatory regions defined here^{4,7} with the nucleotide sequence of *his4*⁸, a gene that is co-regulated with *his3*. The *his3* sequence between nucleotides -44 and -31, TATATAAAGTAATG, strongly resembles the *his4* sequence TATATAATAGATATG, which is located 46-60 bp before the mRNA start point⁸. These sequences contain 12 out of 14 identical base pairs if a single base pair is allowed to 'loop out'. With regard to the upstream regulatory element, the *his3* sequence between

-97 and -90, ATGACTCT, shares 7 out of 8 base pairs with the *his4* sequence ATGACTAT located between -86 and -79. Furthermore, highly conserved variants of this sequence are found at *his3* sequences -139 to -132 (ATGCCTCG) and -178 to -171 (ATTACTCT) and *his4* sequences -137 to -130 (GTGACTCA) and -181 to -174 (GTGACTCC). This striking sequence homology supports the view that these regions are important in *his3* regulation.

Either of two simple models could account for the increased *his3* expression in conditions of amino acid starvation. In one, the gene interacts with positive regulatory factors that stimulate expression above the basal level; in the other model, the *his3* gene interacts with negative regulatory factors that maintain the basal level, and *his3* induction occurs by the inactivation of these factors. Proof of a positive and/or negative mode of *his3* regulation depends on the properties of purified regulatory factors with respect to the appropriate target DNAs. Nevertheless, evidence for the nature of *his3* regulation can be inferred from the phenotypes of regulatory mutations at the *his3* locus.

By definition, mutations at a positive regulatory site would result in basal levels of *his3* expression in all growth conditions. Indeed, five mutations (deletions 20, 22, 23, 24 and 28) have such a phenotype—this strongly suggests that at least some of the sequences deleted by these mutations comprise a positive regulatory site. The other four regulatory mutations result in constitutive expression below the basal level; thus it is difficult to assess their phenotype in terms of positive or negative control.

As for the possibility of negative control, so far I have obtained no *his3* mutations that result in constitutive *his3* expression at the induced level; these would be expected if the gene contains a site of negative regulation analogous to the

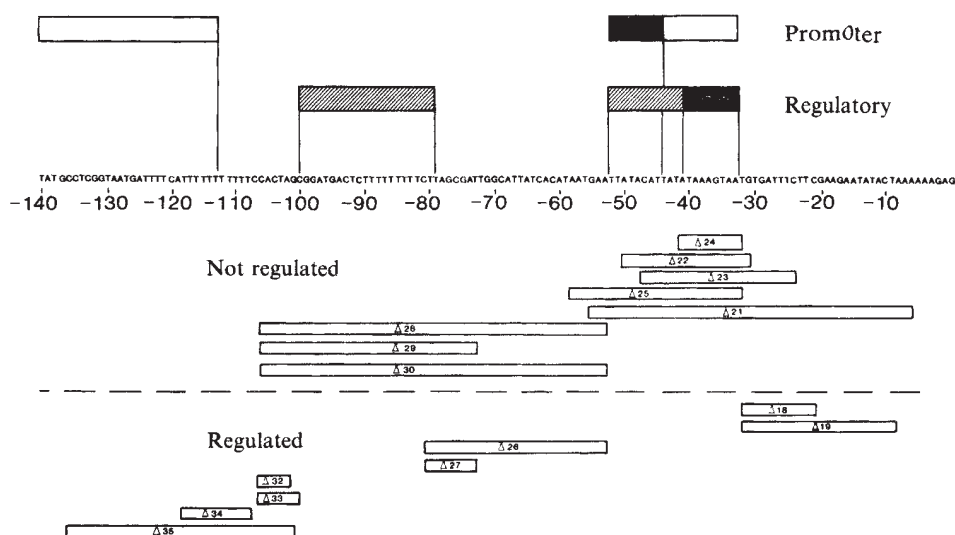


Fig. 2 *his3* promoter and regulatory elements. The open boxes on the top line indicate the extent of promoter elements defined previously^{5,6} and the shaded boxes on the second line indicate the location of positive regulatory elements described here (the black regions within these boxes indicate sequences that are essential but not necessarily sufficient for promoter or regulatory function). These elements are placed with respect to the coding strand of the 5' flanking region (nucleotides +1 to -140 defined with respect to the start of transcriptional initiation). The mRNA start was previously defined to an accuracy of ± 1 bp⁷. The location of the deletion mutations used in this study are indicated below the DNA sequence. Deletions above the broken line do not allow for properly regulated *his3* expression, while the deletions below the line are regulated correctly.

operator of the *Escherichia coli* lactose operon. Nevertheless, it is possible that the five mutations expressing *his3* constitutively at the basal level could delete a negative regulatory site and simultaneously reduce promoter activity threefold. However, the principal advantage of deletion analysis is the ability to eliminate DNA sequences and hence genetic functions. For example, nine mutations described here eliminate regulatory behaviour, and 30 mutations (described in refs 5, 6) eliminate detectable *his3* expression. Thus, it seems unlikely that the five critical deletions fortuitously result in the same quantitatively minor defect, particularly considering the fact that nine deletions having full or partially reduced *his3* levels in normal conditions regulate the gene properly. Therefore, the

mutants strongly support a positive control mechanism for *his3* expression as opposed to a negative one.

The data presented here suggest that the *his3* regulatory region consists of two separable positive control sites, each of which can be destroyed without apparently impairing promoter function. The conclusions represent the simplest formal description of the data, but have no bearing on the molecular mechanism by which *his3* expression is modulated in response to amino acid starvation. As induction of IGP activity is accompanied by an increase in *his3* mRNA⁹, it is likely that regulatory sites are involved in transcriptional initiation; this, however, has not been proved.

The key question is the nature of the 'positive regulatory factor'. Some possibilities are: (1) a protein product(s) that binds specifically to the sequence(s) defined as regulatory elements; (2) a conformational or covalent alteration in yeast RNA polymerase II that senses the regulatory sequences; (3) a structural feature of chromatin; and (4) a structural change in the *his3* transcript that affects its stability or translatability. Biochemical analysis of strains harbouring 'regulation-defective' *his3* alleles should be useful for distinguishing among these and other molecular models. Preliminary results with wild-type cells suggest that the chromatin structure and the mRNA structure are unchanged during amino acid starvation (ref. 4 and K.S., in preparation).

In considering molecular models for *his3* regulation, it is worth comparing the results obtained here with those of an analogous situation in *E. coli*. Amino acid starvation of *E. coli* results in increased expression of amino acid biosynthetic genes and decreased expression of those encoding stable RNA species such as the tyrosine-inserting tRNA. A mutation of this tRNA gene that changes sequences between the downstream promoter element (the Pribnow box) and the start of transcription retains promoter function but is regulated incorrectly¹⁰. Further, *in vitro* transcription of this gene using purified components suggests that regulation of the tyrosine tRNA promoter is mediated by the interaction of *E. coli* RNA polymerase with guanosine tetraphosphate¹¹. It is striking that the *his3* regulatory site between -32 and -41 also maps between (or overlaps with) the TATA box region and the mRNA start. Whether this means that regulation in yeast occurs by a mechanism similar to that used by *E. coli* remains to be determined.

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Table 2 Structures and phenotypes of *his3* derivatives

Fragment	<i>his3</i> allele	Upstream end point	Junction sequence	Downstream end point	Basal level	Induced level
Sc2605	Wild type	—	—	—	1.0	3.0
Sc2854	Δ18	-32	RI	-21	0.5	1.5
Sc2857	Δ19	-32	RI	-32	0.7	2.0
Sc3101	Δ20	-43		-31	1.1	1.0
Sc3102	Δ21	-56		-6	0.2	0.2
Sc3110	Δ22	-50		-31	1.2	1.3
Sc3111	Δ23	-47		-24	0.8	1.0
Sc3112	Δ24	-41	CC	-32	1.1	1.4
Sc3113	Δ25	-58	TCC	-32	0.2	0.2
Sc3121	Δ26	-80	RI	-53	0.9	2.7
Sc3122	Δ27	-80	RI	-73	0.6	1.5
Sc3125	Δ28	-106	RI	-53	1.1	1.0
Sc3126	Δ29	-106	RI	-73	0.2	0.3
Sc3129	Δ30	-119	RI	-53	0.2	0.1
Sc3138	Δ31	-106	RI	-109	1.0	3.1
Sc3159	Δ32	-106		-102	0.8	2.9
Sc3160	Δ33	-106		-100	1.2	3.2
Sc3161	Δ34	-118		-107	0.2	0.6
Sc3165	Δ35	-136		-101	0.5	1.2
Sc2884	Δ38	-80	RI	-32	<0.1	<0.1

All Sc fragments (first column) are derivatives of Sc2605 (see Fig. 1 legend). The *his3* deletion allele numbers and the end points with respect to the start of transcription (determined elsewhere by direct DNA sequencing) are indicated (columns 2 and 3). In some cases, there are junction nucleotides at the site of deletion (RI, *EcoRI* octanucleotide linker, 5'-GGAATTC-3'). For each *his3* allele tested, 1 Ura⁺ transformant (containing one integrated copy of the transforming DNA) and 1 His⁺ transformant (in which the *his3*-532 allele is replaced with the transforming *his3* allele) were grown (see Fig. 1 legend for details). These always gave similar results; the values shown represent the averages of the two strains. To obtain the basal IGP levels for a given mutation, strains were grown in complete medium. To obtain enzyme levels in starvation conditions, tryptophan was removed from the medium (see Table 1 legend for the methodology and reliability of the assay).

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Isolation of a mouse pseudo tRNA gene encoding CCA—a possible example of reverse flow of genetic information

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Transfer RNA genes offer an interesting system for studies of gene regulation. From experiments conducted with tRNA genes from eukaryotes, it seems that transcriptional regulatory regions are contained within the mature coding sequence of the DNA^{1–4}. In tRNA genes of some yeast, *Xenopus* and *Drosophila*, intervening sequences located within the anticodon loop have been identified^{5–8}, thus adding a new dimension to tRNA expression. In contrast to the lower eukaryotes, relatively few mammalian tRNA genes have been studied. The genes obtained so far have been identified via cloned heterologous fragments, or labelled tRNA probes^{9–11}. We sought to use synthetic oligodeoxyribonucleotide probes to isolate mouse phenylalanyl tRNA genes. We report here the isolation of a segment of DNA that is completely homologous to our 19-mer probe, as well as 38 nucleotides corresponding to the 3' end of tRNA^{Phe}. The 5' end of the gene is not present in our 1.7 kilobase clone. In addition, this pseudo gene has an encoded CCA. Since CCA is added post-transcriptionally to all known eukaryotic tRNAs for which genes have been identified, we believe this pseudogene to be representative of RNA encoded information going back into DNA.

We chose to isolate a mouse gene coding for tRNA^{Phe} by using as a probe a synthetic oligodeoxyribonucleotide 19-mer complementary to a portion of the known mammalian tRNA^{Phe} sequence^{12,13} (see Fig. 1a). The strategy utilized was the screening of a partially digested mouse DNA library inserted at the *Hind*III site of pBR322. This plasmid library was used to transform *Escherichia coli* and selection was made for ampicillin resistant colonies. The colonies were screened for the tRNA^{Phe} gene containing inserts as described in Fig. 1 legend. Figure 1b, c shows autoradiographs of colony hybridizations with the synthetic probe. Four putative positives were obtained by the colony screening, two of which gave positive hybridization results on rescreening. These two clones appeared to be siblings as judged by DNA restriction (not shown). One of these plasmids, designated as pMTF3, was chosen for further studies.

Figure 2a presents ethidium bromide stains of restriction endonuclease digestions of pMTF3. Digestion with *Hind*III yielded a 3.4 kilobase pair (kbp) vector band, and 1.2 and 1.7 kbp insert bands. It is not known if the two *Hind*III inserts were due to ligation of partially digested DNA or were the result of ligation of two separate fragments. To test which of the two *Hind*III fragments contained the sequence homologous to the 19-mer probe, the DNA in the gel was transferred to nitrocellulose¹⁴ and then hybridized with the ³²P-labelled synthetic 19-mer probe. These results demonstrated that only the 1.7 kbp *Hind*III fragment contained a sequence homologous to the probe (Fig. 2a). Digestion of pMTF3 with *Ava*I

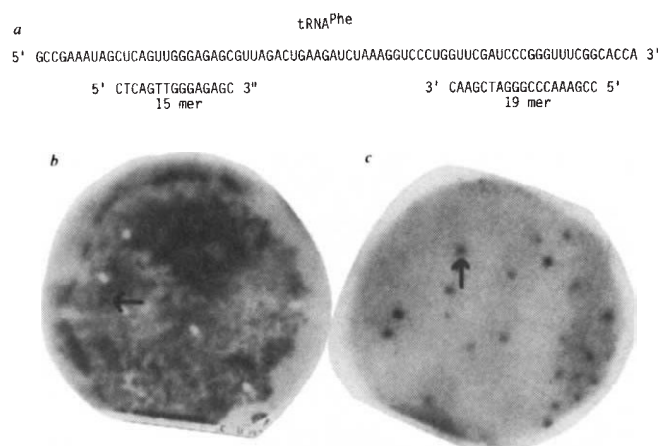


Fig. 1 Colony screening with a synthetic oligonucleotide complementary to tRNA^{Phe}. *a*, The sequence of mammalian tRNA^{Phe}^{12,13} (above) with the 19 base oligodeoxyribonucleotide complementary to the 3' region of the tRNA, and a 15 base oligonucleotide containing the same sequence as the dihydrouridine stem and loop region (below). Both oligodeoxyribonucleotides were synthesized using the phosphotriester method (38). *b*, Examples of colony screening using the synthetic 19 mer as a probe. Approximately 5 µg of mouse BALB/c liver DNA (partially digested with *Hind*III) was ligated with 1 µg of pBR322 DNA digested with *Hind*III and treated with alkaline phosphatase. This DNA mix was used to transform *E. coli* strain MC1061³⁹ from which approximately 1 × 10⁵ transformants were obtained, about 90% of which were tetracycline sensitive. This represents approximately 15–20% coverage of the genome. These colonies were first replicated to dry, sterile nitrocellulose filters. The colonies on nitrocellulose were used to make replicas onto Whatman 541 paper⁴⁰. The nitrocellulose replicas were incubated on L-agar plates containing ampicillin (30 µg ml⁻¹) and used as masters for retrieving positively hybridizing colonies. The Whatman 541 replicas were treated according to Gergen *et al.*⁴⁰, and hybridized with 75 pmol (500 × 10⁶ c.p.m.) ³²P-polynucleotide kinase labelled 19-mer. The hybridizations were carried out overnight at 45 °C in 3–5 ml per filter of 6 × SET (30 mM Tris-HCl pH 7.5, 0.9 M NaCl, 6 mM EDTA), 5 × Denhardt's medium, 0.5% Nonidet P40 following 2–3 hours of prehybridization in the same buffer. The filters were washed in 6 × SET at progressively higher temperatures, beginning with 20 °C, until background hybridization was satisfactorily eliminated⁴¹. The final wash was at 45 °C. After washing, the filters were autoradiographed at –70 °C on Kodak XRP-1 film with a DuPont Lightning Plus intensifying screen. *b*, A small area (approximately 1 cm) corresponding to the region of a positively hybridizing colony was scraped, diluted, replated and rescreened with the 19-mer probe. *c*, Positively hybridizing, isolated single colonies from the rescreening were used for DNA isolations.

(CPyCGPuG) gave three stained fragments that had summated fragment lengths equal to undigested pMTF3. No hybridization with the 19-mer probe was seen in the *Ava*I digest (Fig. 2a). This same lack of hybridization was observed with *Hpa*II (CCGG) and *Sma*I (CCCGG) digests (data not presented). This failure to detect hybridization with the 19-mer probe in the *Ava*I, *Hpa*II and *Sma*I digests is due to the presence of these sites in the middle of the region of complementarity to the 19-mer probe (Fig. 1).

Using standard double and triple restriction endonuclease digestions, a restriction site map for pMTF3 was constructed (Fig. 2b). Since the *Sma*I site (Figs 1a, 2b) is in the centre of the region of complementarity with the 19-mer probe, the DNA sequences including and surrounding this site were determined by the chemical cleavage method¹⁵.

The DNA sequencing results are summarized in Fig. 3. From these data we find a region of perfect homology to the probe. Comparison to the known tRNA^{Phe} sequence (Fig. 3) demonstrates that 38 contiguous nucleotides of the tRNA^{Phe} 3' region are included in the cloned segment. This homology diverges within the region corresponding to the tRNA^{Phe} anticodon loop.

a STAIN

HYBRIDIZATION

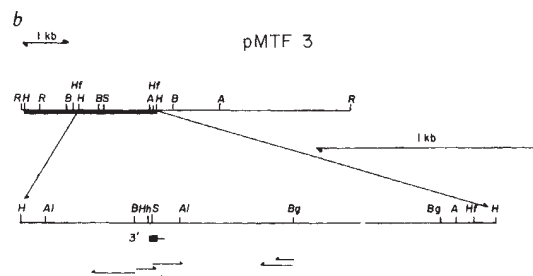
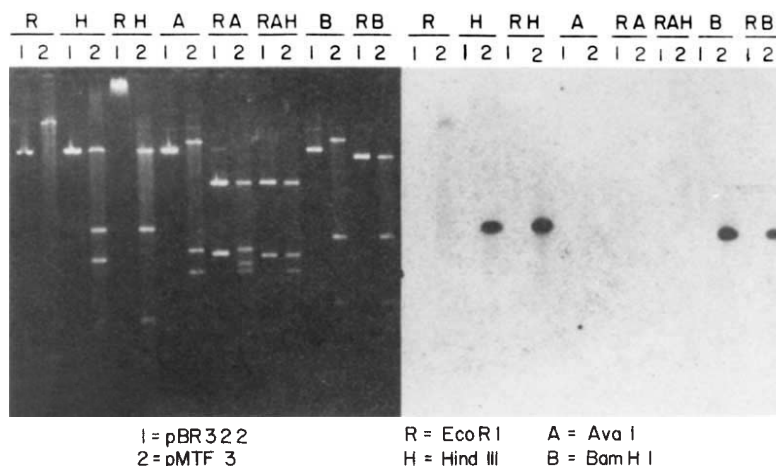


Fig. 2 a, Restriction endonuclease digestions and hybridization of the 19-mer probe to pMTF3. Plasmid DNA from pMTF3 was prepared and purified by ethidium bromide CsCl gradients⁴². This DNA was then treated with various restriction enzymes (as shown) under conditions described by Bethesda Research Laboratories (BRL). Samples were then subjected to electrophoresis through an 0.8% agarose gel. The gel was stained and photographed. Markers of *Hind*III, *Eco*RI and *Hpa*II digested λ DNA were run in adjacent channels (not shown). Transfer of the DNA to nitrocellulose was by the method of Southern¹⁴. Prehybridization of the filter was at 42 °C in 6 $\times$ SSC plus 5 $\times$ Denhardt's medium for 12 hours³⁴. Hybridization was for 36 hours with 6 $\times$ SET, 5 $\times$ Denhardt's, and 50 $\times$ 10⁶ c.p.m. ³²P-labelled 19 mer. After hybridization, the filter was washed four times for 1 hour each at 42 °C in 6 $\times$ SSC and exposed to Kodak XAR-5 film at -70 °C with DuPont Lightning Plus intensifying screens. The faint hybridization of the 19-mer probe to the *Eco*RI digest was the result of poor transfer from the gel to nitrocellulose. b, A restriction site map of pMTF3. Shown is the pBR322 vector linearized at its *Eco*RI site and emphasizing the *Hind*III inserts. The position and polarity of the tRNA^{Phe} pseudogene sequence is shown (■). Also depicted are the restriction sites used for labelling and the direction of DNA sequencing. Restriction sites shown are R, *Eco*RI; H, *Hind*III; B, *Bam*HI; S, *Sma*I; A, *Ava*I; Al, *Alu*I; Bg, *Bgl*II; Hf, *Hinf*I; and Hh, *Hha*I.

No sequence completely homologous to the 5' end of tRNA^{Phe} was found within 100 base pairs from this point of divergence (Fig. 3).

In an attempt to localize the 5' end of tRNA^{Phe}, a synthetic oligodeoxyribonucleotide containing the dihydrouridine stem and loop region sequences of tRNA^{Phe} (Fig. 1) was chemically synthesized. This 15-mer was used to probe DNA from pMTF3 as described above. The only hybridization observed generated a weak signal with a restriction fragment which maps to the 3' side (downstream) of the region of the 19-mer homology (data not presented). DNA sequence analyses of this region did not demonstrate any region of complete homology with the 15-mer probe nor with the known tRNA^{Phe} sequence.

The presence of the 3' end of tRNA^{Phe} and the apparent absence of the 5' end suggests a pseudogene. The presence of an encoded 3' terminal CCA in our cloned segment is also quite unusual. An examination of published sequences for over 50 nuclear tRNA genes from such diverse higher eukaryote sources, such as, *Bombyx*, *Drosophila*, *Xenopus*, rat and man, reveals no encoded CCAs^{7,10,11,16-23}. Furthermore, none of the 22 mouse⁴⁵ or human²⁴ mitochondrial tRNA genes encode CCAs. All available evidence suggests CCAs are added post-transcriptionally in eukaryotes. Several other interesting features of the

cloned mouse DNA segment in pMTF3 must be considered. The position at which the cloned sequence diverges from the known tRNA^{Phe} sequence is one base pair off from the site at which an intervening sequence has been localized in some eukaryotic tRNA genes⁵⁻⁸. The 5' end of tRNA^{Phe} has some points of weak homology to pMTF3 in the region sequenced. These sequences are noted in Fig. 3. We do not know if these similarities represent divergence in the 5' region or if they are random. The nucleotide sequence presented in Fig. 3 has been analyzed for tRNA-like secondary structures using the computer program developed by Staden²⁵. No structures capable of forming stable tRNA secondary structures with the appropriately conserved nucleotides were predicted by this search.

In screening 15–20% of the mouse genome (see Fig. 1 legend), we obtained a single tRNA^{Phe} related recombinant (pMTF3). This yields a minimum estimate of five tRNA^{Phe} related sequences per haploid genome. We have not yet isolated the complete tRNA^{Phe} gene. This could be due to a lower than predicted gene copy number, or a bias in our cloning approach.

To determine if the cloned tRNA^{Phe} pseudogene was indeed derived from mouse, the *Hind*III 1.7 kbp insert fragment was used as a probe to mouse genomic DNA. The results presented in Fig. 4 show a smear of hybridization spread over the entire

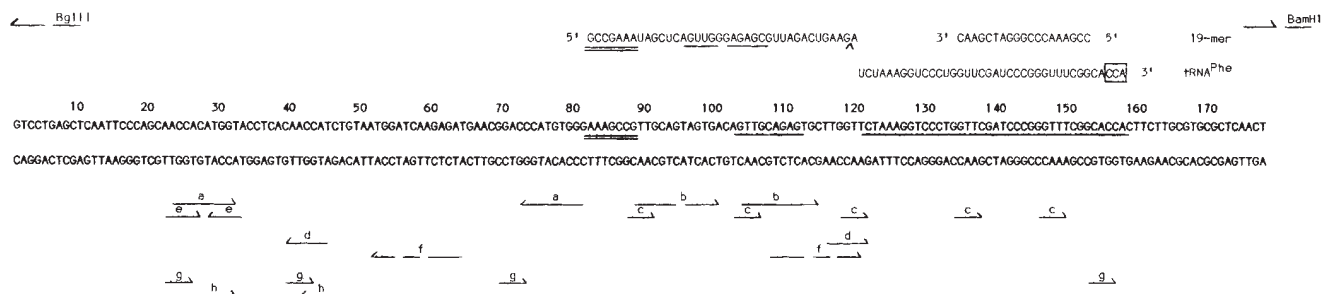


Fig. 3 DNA sequence of pMTF3 in the region of 19-mer homology. The pMTF3 sequence is aligned with the known mammalian tRNA^{Phe} sequence using the tRNA^{Phe} CCA as the start point. Nucleotides in the pMTF3 sequence which are present in the corresponding position of tRNA^{Phe} are underlined. Note that the 5' terminal tRNA^{Phe} sequence GCCGAAA is present as an inverted segment at the same relative position in pMTF3. Other areas of interest, such as direct (→→), inverted (→←), palindromic repeats (←→), and the position at which an intervening sequence would be expected to occur (Δ) are indicated.

genomic restriction profile for each of the enzymes or combinations tested. Hybridizations done under identical stringent conditions with mouse ribosomal gene probes yielded specific banding patterns²⁶. The broad hybridization distribution of the 1.7 kbp. *Hind*III band to numerous genomic fragments indicates the presence of repetitive elements in the clone. In a separate experiment (not shown), mouse genomic DNA was hybridized to restriction digests of pMTF3. The data indicate that there are repeated sequences located on both sides of the pseudo gene. The vector DNA showed no homology to mouse. It is not known whether these elements are tRNA genes or members of a mouse family of repeated sequences, or both.

The origin and functional significance, if any, of all pseudogenes is of interest. In *E. coli* several pseudogene sequences for tRNA^{Tyr} which contain partial, but non-degenerate homology with the mature coding sequences are found distal to the mature coding sequences²⁷. Some biological function is suggested since they are stably maintained in the chromosomes of several *E. coli* K12 strains²⁸ and are included in the tRNA^{Tyr} containing primary transcripts²⁹. Little is known concerning any possible functional role of eukaryotic pseudogene sequences. The *Xenopus* 5S RNA pseudogenes are 85% homologous with the 5S genes³⁰. In one report the pseudogene sequence was observed to be transcribed following injection of the cloned DNA segment into *Xenopus* oocytes³¹. It is not clear whether these sequences are transcribed *in vivo*. Transfer RNA pseudogenes which share degenerate or incomplete homologies with the mature coding sequences, have been described for *Drosophila* tRNA^{met} (ref. 20) and rat tRNA^{Gly} and tRNA^{Glu} (ref. 23). Unlike the tRNA^{Phe} pseudogene sequence reported here, neither the *Drosophila*, nor rat pseudogene sequences contain an encoded CCA. Mammalian pseudogenes for globin, tubulin and immunoglobulin have been reported which have structural and sequence features suggesting an RNA origin³²⁻³⁴.

Any model used to explain the origin of the tRNA^{Phe} pseudogene sequence must take into account the facts that this sequence is completely homologous with 38 3'-terminal nucleotides of tRNA^{Phe} and has an encoded CCA. This region of homology is more extensive than the tRNA priming sequences found in retroviral DNAs (~18 nucleotides)³⁵, although we cannot eliminate a retroviral origin for this pseudogene sequence. Since we do not know the structure of the tRNA^{Phe} gene itself, we can only speculate based on analogy with other eukaryotic tRNAs that the CCA is added post-transcriptionally, and therefore is not encoded.

Fig. 4 Hybridization of pMTF3: *Hind*III A band to mouse genomic restriction digests. DNA from a male BALB/c kidney was prepared and digested with various combinations of *Eco*RI, *E*; *Hind*III, *H*; or *Bam*HI, *B*. Completion of the digests was verified using λ DNA as an indicator. The samples were then subjected to electrophoresis through 0.8% agarose, stained and photographed. Markers of lambda *Hind*III and *Eco*RI were run in adjacent channels. The DNA in the gel was then transferred to nitrocellulose by the method of Southern¹⁴. The probe was prepared by first digesting pMTF3 with *Hind*III, separating out the fragments depicted in Fig. 2, and recovering the *Hind*III A band via electroelution. The recovered band (0.5 μ g) was then nick translated⁴³ with [α -³²P]dCTP. Hybridization of the probe to the filter was by the method of Wahl *et al.*⁴⁴, with modifications of Reilly *et al.*²⁶. The final wash was at 65 °C in 0.2 $\times$ SSC. Autoradiography was as described for Fig. 2.



The presence of an encoded CCA suggests that the information of the mature tRNA was reverse transcribed and integrated into the mouse genome. A similar phenomenon is suggested for tubulin³³ and immunoglobulin pseudogenes³⁴. We do not know the mechanisms by which RNA characteristics, such as poly(dA) stretches, attached to intron-less coding regions (as seen in tubulin and globin pseudogenes) or the encoded CCA in pMTF3 arose. To explain the origin of such pseudogene sequences, mechanisms of the reverse transcription and genomic integration of the segments must be understood. In the absence of any clearly identified primer, we find it difficult to understand how a reverse transcript of only the 3' end of tRNA^{Phe} arose. With regard to integration of this pseudo-gene into the genome, repeated family sequences could be involved. The recent discovery of mammalian nuclear extrachromosomal circular DNA containing *Alu* family sequences suggests a possible intermediate³⁷. In the case of several small nuclear RNA pseudogenes, the pseudogene sequences are flanked by short (16–19 bp) direct repeats³⁶. We do not observe these types of repeats flanking the tRNA^{Phe} pseudogene, although pMTF3 does contain repetitive sequences (Fig. 4). Any further insight into the origin of this pseudogene must await isolation and analysis of the complete tRNA^{Phe} gene.

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Effect of interferon on protein glycosylation and comparison with tunicamycin

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The present wide interest in the mechanism of action of interferon (IFN) stems from its antiviral and anticancer potential. Possibly one of the more interesting insights into the antiviral action of IFN was recently described by Maheshwari *et al.*¹. These authors, including one of us (K.O.), reported that IFN-treated cells release viral particles with low infectivity, and that this low infectivity seems to be related to the reduced amount of membrane protein glycosylation. The implications of this suggestion are exciting because inhibitors of glycosylation have antiviral properties, and also selectively kill transformed cells (see ref. 2 for review). An effect of IFN on the synthesis and cell-surface expression of glycoproteins could have an important role in its antiviral, anticancer and immunoregulatory actions. We have examined, for the first time, the effect of IFN on glycosylation of cellular proteins. We used murine fibroblasts (L-cells and NIH-3T3), plasmacytoma cells (P3×63 Ag8) and vesicular stomatitis virus (VSV) to compare the action of IFN with tunicamycin (TM), a known inhibitor of glycosylation². Our results indicate that IFN does not inhibit the glycosylation of cellular or viral glycoproteins. Cells treated with IFN do not produce nonglycosylated species of glycoproteins nor the 'glucose/glycosylation regulated proteins'³ (GRPs), in contrast to cells treated with tunicamycin⁴.

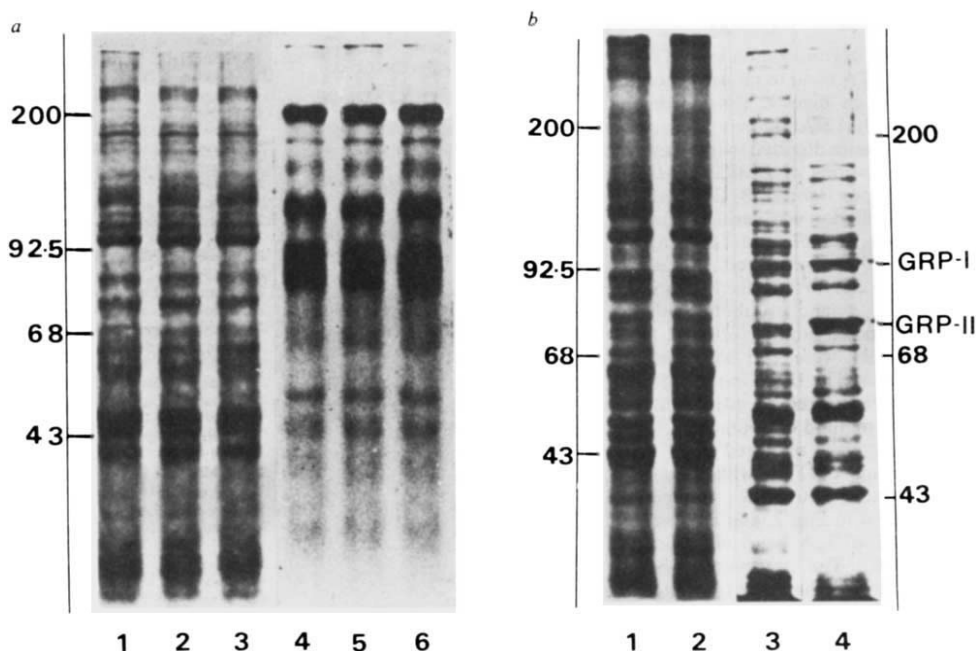
Fibroblasts were grown in Eagle's minimal essential medium (MEM) as described by Maheshwari *et al.*^{1,5-8}, and plasmacytoma cells in Dulbecco-Vogt⁹ modified Eagle's medium containing high glucose (4,500 mg l⁻¹). Cells were infected with VSV at a multiplicity of 0.5–10 plaque forming units (PFU) per cell as previously described^{1,5-8}, and radiolabelled substrates were added 3 h post-infection. Plaque formation was determined according to standard assay procedure¹⁰ with confluent cultures of L-cells.

First, we compared the effects of IFN and TM on the glycosylation of cellular protein by monitoring the rate and total incorporation of D-[U¹⁴C]glucosamine and D-[2³H]mannose into the total trichloroacetic acid-insoluble fraction of the cell. The results of a representative 48-h incorporation experiment are shown in Table 1a. It is apparent that none of the concentrations of IFN were effective in inhibiting glycosylation of cellular protein, in marked contrast to TM. Similarly, no differences were found in the rate of incorporation into cultures monitored at hourly intervals for up to 3 h. Likewise, IFN had no effect on the glycosylation of specific proteins as evident by inspection of the fluorogram shown in Fig. 1a. This conclusion is also supported by the finding that IFN-treatment does not induce the synthesis of the GRPs, in contrast to cells treated with TM (Fig. 1b, and refs 4, 11).

To investigate further the effect of IFN on the glycosylation of specific cellular glycoproteins, metabolically labelled fibronectin (FN) and immunoglobulin G (IgG) were isolated by immunoprecipitation and analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Inspection of the fluorograms in Fig. 2 shows that IFN does not decrease the glycosylation of either protein as shown by similar incorporation of radiolabelled carbohydrate precursor and by lack of shift in electrophoretic mobility as would be expected if the proteins were depleted of carbohydrate¹².

To determine if treatment of cells with IFN had any effect on the size or structure of the oligosaccharide chains of an asparagine-linked cellular glycoprotein, glycopeptides of FN were isolated from control and IFN-treated 3T3 cells and subjected to exhaustive digestion with pronase. The glycopeptides were analysed by Bio-Gel P4 filtration, treatment with *endo*-β-N-acetylglucosaminidase H (*endo* H), and by their

Fig. 1 a, Effect of IFN on glycosylation of plasmacytoma cellular proteins. Lanes 1, 2 and 3 represent ¹⁴C-leucine (2 μCi ml⁻¹, specific activity 2 Ci mmol⁻¹) labelled cells incubated with 0, 30 and 250 U ml⁻¹ of mouse fibroblast IFN (specific activity >4 × 10⁷ mouse reference units per mg protein) respectively. Lanes 4, 5 and 6 represent parallel cultures treated with similar amounts of IFN, but labelled with ¹⁴C-D-glucosamine (5 μCi ml⁻¹, specific activity 238 mCi mmol⁻¹). Cells were preincubated with IFN for 16 h, then radioactive precursors were added and incubation was continued for 48 h. SDS-PAGE and fluorography were performed as previously described^{23,24}. Protein was determined by the Lowry procedure²⁵ with bovine serum albumin standards. Similar results were obtained with IFN concentrations up to 1,000 U ml⁻¹, at 24 and 36 h, or with IFN purchased from Lee Biomolecular Research Laboratories. b, Effect of IFN and TM on the induction of the 'glucose/glycosylation regulated proteins'. Mouse L-cells were labelled with ¹⁴C-leucine and incubated with IFN as described in Fig. 1 legend. Cells were pretreated for 6 h with TM before addition of ¹⁴C-leucine. Lanes 1 and 2 represent cells treated with 0 and 30 U ml⁻¹ IFN, respectively, and lanes 3 and 4 represent cells treated with 0 and 1.0 μg ml⁻¹ TM, respectively. Similar results were obtained with IFN concentrations up to 1,000 U ml⁻¹.



interaction with concanavalin A (Con A)-Sepharose. We found that glycopeptides from both the control and IFN-treated cells were resistant to *endo* H, reacted with Con A, and co-eluted on Bio-Gel columns with a molecular weight ($\sim 2,300$) characteristic of the complex-type oligosaccharide (results not shown).

The above experiments provide strong evidence that IFN does not significantly alter the carbohydrate moieties of asparagine-linked cellular glycoproteins.

To determine if VSV produced in the presence of IFN contain surface carbohydrates, viral particles isolated from control and IFN-treated cultures were examined with respect to their specific binding to Con A-Sepharose. If viral particles released from IFN treated cells are depleted of the carbohydrate moiety on glycoprotein G, then they should not bind to Con A.

Table 1 Effect of tunicamycin and interferon on glycosylation of cellular protein (a) infectivity of VSV (b) and binding of VSV to concanavalin A-Sepharose (c)

a. Glycosylation			
Tunicamycin Precursor	[TM] ($\mu\text{g ml}^{-1}$)	c.p.m. per μg protein	% Inhibition
^{14}C -leucine ($2 \mu\text{Ci ml}^{-1}$)	0	1,123	0
	0.05	1,309	0
	0.10	1,174	0
	0.50	1,060	12
	1.00	929	23
^3H -mannose ($5 \mu\text{Ci ml}^{-1}$)	0	422	0
	0.05	437	0
	0.10	316	25
	0.50	241	43
	1.00	148	65
Interferon			
Precursor	[IFN] (U ml^{-1})	c.p.m. per μg protein	% Inhibition
^{14}C -leucine ($2 \mu\text{Ci ml}^{-1}$)	0	1,229	0
	30	1,204	0
	100	1,171	3
	250	1,182	3
	500	1,165	5
^3H -mannose ($5 \mu\text{Ci ml}^{-1}$)	1,000	1,110	9
	0	479	0
	30	460	4
	100	461	4
	250	453	5
^{14}C -glucosamine ($2 \mu\text{Ci ml}^{-1}$)	500	446	7
	1,000	437	9
	0	934	0
	30	941	0
	100	926	1
	250	911	2
	500	873	7
	1,000	860	8
b. Infectivity			
Conditions	PFU ml^{-1}	c.p.m. ml^{-1}	PFU c.p.m.
Control	9.48×10^8	3.9×10^4	2.43×10^4
IFN-treated	9.57×10^8	3.9×10^4	2.45×10^4
c. Binding to Con A			
Conditions	c.p.m. added	Concanavalin A	
		% Retained	% Retained
		(-) α -methyl-D-mannopyranoside	(+) α -methyl-D-mannopyranoside
Control	210,000	97 ± 2	2
IFN-treated	210,000	96 ± 2	2
TM-treated	210,000	17 ± 5	2

a, Test cultures were preincubated for 6 and 16 h with TM or IFN, respectively, before the addition of radiolabelled precursors. Cells were labelled for 24 h with leucine ($2 \mu\text{Ci ml}^{-1}$), glucosamine ($2 \mu\text{Ci ml}^{-1}$) and mannose ($5 \mu\text{Ci ml}^{-1}$), extracted once with 10% TCA and twice with 5% TCA. The precipitate was dissolved in 0.5 M NaOH, neutralized with HCl, diluted in Aquasol (NEN) and counted by liquid scintillation spectrometry. A representative experiment is shown; however, the experiment was performed several times by different individuals with similar results. In addition, incorporation of radiolabelled precursors was monitored for 48 h with IFN, and no inhibition of incorporation was observed. b, Cells were pretreated with IFN (30 U ml^{-1}) for 16 h. Virus production, purification and infectivity assay are described above in the legends to the figures. c, The amount of ^{14}C -leucine radioactivity associated with the purified viral particles was determined, and equal number of counts were applied to gels to show that these fractions contained equivalent amounts of virus-specific proteins (see Fig. 3). The total protein in these fractions was shown to be the same by the Lowry procedure. As IFN treatment (30 U ml^{-1}) of the L-cells inhibited viral production by 10–20-fold, we had to use 10 times more IFN-treated than control cultures to obtain a similar yield of viral particles. Cells were treated with $1.0 \mu\text{g ml}^{-1}$ of TM. The binding to Con A was performed according to the procedure of Gibson *et al.*¹⁵, except that the lectin-coupled gel beads were in suspension.

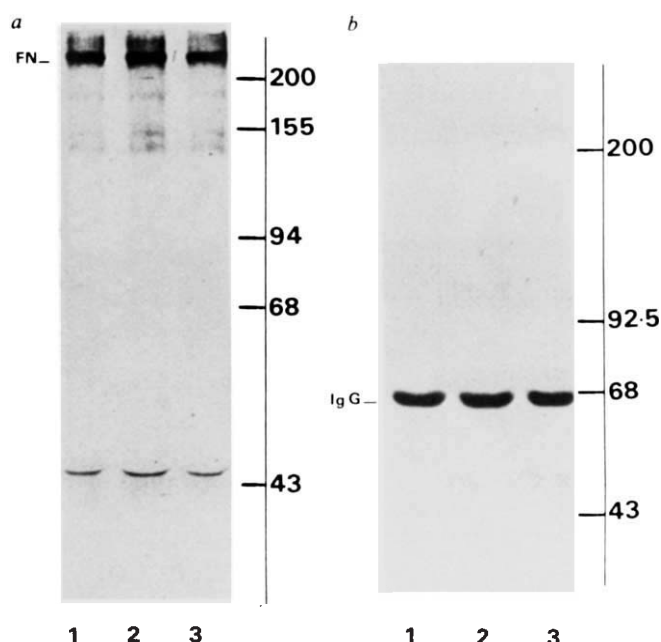


Fig. 2 Effect of IFN and TM on glycosylation of specific cellular proteins. a, 3T3 cells were treated with IFN and labelled with ^{14}C -D-glucosamine as described in Fig. 1 legend. FN was isolated by immunoprecipitation as described previously³. Lanes 1, 2 and 3 represent 0, 30 and 250 U ml^{-1} IFN respectively. b, Plasmacytoma cells were treated with IFN and labelled with ^{14}C -D-glucosamine as described in Fig. 1 legend. IgG was isolated by mixing 1.0 ml of the conditioned, cellular debris-free medium with $40 \mu\text{l}$ of goat anti-mouse IgG ($160 \mu\text{g}$ protein; Cappel) and incubated for 60 min at room temperature. The precipitate was collected by centrifugation, and washed twice with phosphate-buffered saline. Lanes 1, 2 and 3 represent 0, 30 and 250 U ml^{-1} IFN. Similar results were obtained with IFN concentrations up to $1,000 \text{ U ml}^{-1}$.

However, we found that $>94\%$ of the purified viral particles, from both control and IFN-treated cultures, were specifically adsorbed to Con A as shown in Table 1c. As this lectin binds to mannose-containing oligosaccharides, this suggests that glycoprotein G is glycosylated in the presence of IFN. This was confirmed by direct fluorographic analysis of ^{14}C -D-glucosamine labelled viral particles (Fig. 3, lanes 4–6).

Previous studies have shown that the absence of the carbohydrate moiety caused a drastic reduction in the amount of G protein incorporated into the released VSV particles^{13–15}. Because the infectivity of VSV is dependent on this protein^{15–21}, this was used as an indirect measure of G present on the released virions. An equivalent number of viral particles, isolated from control and IFN-treated cultures, was spread on a lawn of L-cells, incubated for 72 h at 37°C and scored for PFU. The particles produced in the presence of 30 U ml^{-1} of IFN (the concentration used by Maheshwari *et al.*^{1,5–8}) had the same specific infectivity as those produced by untreated control cultures (Table 1b). However, IFN drastically inhibited (10–20-fold) the production of VSV, and the viability of the IFN-treated, VSV-infected cells was not significantly compromised compared with infected cells not treated with IFN. Concentrations of IFN $\geq 150 \text{ U ml}^{-1}$ did decrease infectivity, but had no effect on glycosylation. This finding suggests: (1) that IFN treatment does not selectively inhibit the incorporation of G into viral particles, and (2) that the primary mechanism of IFN's antiviral action is by inhibition of viral production as previously reported²².

To demonstrate directly that the released virions are not defective in glycoprotein G, viruses were isolated from control and IFN-treated cultures and analysed by SDS-PAGE. The four viral proteins (L, G, N and M) are clearly visible in the fluorogram of ^{14}C -leucine-labelled viruses produced in either the presence or absence of IFN (Fig. 3, lanes 1–3), and the relative amounts of the four proteins are unaffected by IFN treatment. Careful inspection of the published data of

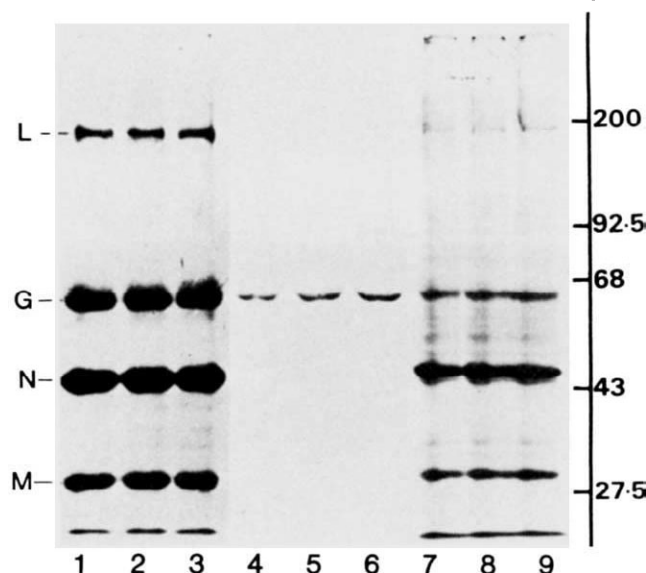


Fig. 3 Effect of IFN on protein G. L-cells were treated with IFN and metabolically labelled as described in Fig. 1 legend. To prepare purified viruses, the culture media were collected and centrifuged at 10,000g for 10 min to remove large cellular debris. The supernatants were centrifuged at 48,000g for 2 h. The pellets containing the virus were further purified by the sucrose density gradient procedure of Leavitt *et al.*¹³. The viruses were resuspended in MEM and centrifuged for 1 h at 100,000g to remove the sucrose. The viruses were then resuspended in MEM (without supplements) containing 100 µg ml⁻¹ antifibronectin coupled to Sepharose 4B gel beads and incubated for 1 h at 5 °C on a rotatory mixer. The gel beads with the fibronectin containing membrane particles were removed by centrifugation at 10,000g for 10 min. The purified virus particles were stored in MEM at -70 °C, or immediately analysed by SDS-PAGE. Lanes 1, 2 and 3 represent the highly purified viral particles from cells treated with 0, 30 and 250 U ml⁻¹ IFN. Glycoprotein G (lanes 4-6) was immunoprecipitated from 1 ml of a 1% Triton X-100 extract of viral particles (100 µg protein) by the addition of 50 µg of affinity-purified anti-G, and processed as described in Fig. 2 legend. Lanes 7-9 represent the crude viral particles from cells treated with 0, 30 and 250 U ml⁻¹ IFN.

Maheshwari *et al.* (ref. 1, Fig. 1) shows that one likewise cannot make a strong case for a significant and specific decrease in glycoprotein G, contrary to their previous reports^{6,7}.

The results shown in lanes 1-3 of Fig. 3 are with viral particles that have undergone extensive purification, hence it is possible that virus with altered G protein was preferentially lost during purification. This possibility was evaluated by two different approaches. First, we determined the rate of G protein synthesis and glycosylation by pulse labelling with ¹⁴C-leucine, ³H-mannose or ³H-glucosamine for 1 h at 3, 6 and 9 h post-infection of L-cells. Cells containing the newly synthesized viral protein were homogenized and analysed by SDS-PAGE. The polypeptide band corresponding to the G protein was cut from the gel, dissolved in 30% hydrogen peroxide and the radioactivity determined by liquid scintillation spectrometry. There was no apparent difference in the specific activity (¹⁴C c.p.m./³H c.p.m.) of carbohydrate precursor incorporation in the presence or absence of IFN (30-500 U ml⁻¹). The second approach involved the rapid isolation of viral particles without extensive purification. Media from VSV-infected cells, cultured in the presence or absence of IFN (30-500 U ml⁻¹), was centrifuged at 10,000g for 10 min, and the resulting supernatant at 100,000g for 30 min. The pellets containing the virus were homogenized and equal amounts of protein were electrophoresed. The fluorograms are shown in lanes 7-9 of Fig. 3. It is apparent that similar results are obtained with crude and highly purified viral preparations.

The results presented here show that IFN does not inhibit the glycosylation of asparagine-linked glycoproteins as previously reported¹. When [2-³H]mannose was used as an alternative labelling substrate for glycoproteins, similar results were obtained. Therefore, the results obtained with ¹⁴C-D-glucosamine are not experimental artifacts. We conclude that

the antiviral, antitumour and immunomodulatory actions of IFN are probably unrelated to glycosylation.

The present study does not agree with earlier reports by Maheshwari *et al.* claiming that IFN is an inhibitor of glycosylation¹ and that VSV released from IFN treated cells is deficient in glycoprotein G^{6,7} and, as a consequence, is less infectious than virions released by untreated cells^{1,5-8}. The basis for the apparent discrepancies in results are not immediately obvious as we used what we believe to be the same cells, virus and IFN as the previous investigators, and as we also used the same general procedures. Also, we were able to repeat the above results with a similar preparation of IFN obtained from Lee Biomolecular Research Laboratories. There is a major difference in experimental approach relative to viral purification; however, the apparent contradictions cannot be due to the isolation procedure as we were unable to repeat their findings even when we used less pure viral preparations. It is also important to point out that Maheshwari and Friedman^{1,5-8} never studied the effect of IFN on the synthesis of host cell glycoprotein. Also, we have no information relevant to their finding that IFN inhibits the transfer of N-acetylglucosamine from UDP-N-acetylglucosamine into glycolipids¹.

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Corrigenda

In the letter 'Crystal structure of a microbial ribonuclease, RNase St' by K. T. Nakamura *et al.*, *Nature* **299**, 564-566 (1982), four residues are shown incorrectly in Table 1. In RNase St, residue 212 should be 'Q', in RNase Ms residue 32 should be 'D', in RNase Ba residue 49 should be 'K' and in RNase U₂ residue 75 should be 'D'.

In the article 'Fossil mammals and artefacts from the Middle Awash Valley, Ethiopia' by J. E. Kalb *et al.*, *Nature* **298**, 25-29 (1982), the acknowledgements section should include thanks to the Boise Fund (Oxford University) for supporting one of the authors (Dr P. Whitehead).

MATTERS ARISING

The homing mechanism of pigeons

THE review by Gould¹ misrepresents essential aspects of our knowledge about pigeon navigation. Within the limited space available, I cannot substantiate this for the whole article, but can only enumerate the most drastic shortcomings concerning my own work.

In aviary (palisade) experiments: (1) The related papers²⁻⁴ primarily deal with recoveries which comprise sufficiently large numbers (totalling 691!) to prove statistically significant differences between many of the differently treated pigeon groups. Gould discards all these data and considers only initial bearings, although it has been explained why those data are much less reliable (ref. 4, pp. 215-216). (2) Gould's Fig. 2 is meaningless (i) because of the generally restricted value of those initial bearings, (ii) because the semi-louvred palisade used at first³ cannot be directly compared with the open aviary (ref. 4, p. 216), (iii) because the figure mixes different release and home sites in an unbalanced way, and (iv) because it does not even coincide with the published data^{2,3}.

(3) Gould lumps the data of two 'glass and open lofts' and two 'wood and louvered lofts' irrespectively of the orientation of the corridors open to airflow although just this alignment has been shown to influence the pigeons' behaviour⁴. (4) Gould's site-by-site calculations of angular deviations of experimentals from controls (Table 1) conceal rather than disclose the net homeward orientation, as the relation to home is abolished. (5) "These effects" (of the hybrid aviaries) "are generally interpreted as disrupting the pigeon map sense." I wonder where Gould read this. I stressed the influence of the corridors on the preferred compass direction only, but not on homeward orientation, the part requiring a functioning 'map'.

(6) Real differences in homeward orientation have been found between pigeons from completely open or louvered aviaries and much poorer oriented pigeons from aviaries completely shielded with glass walls⁴. These findings, however, primarily concern the "small proportion" of recoveries (sample sizes 58 and 63) and thus are neglected by Gould. (7) The corridor-aviary results⁴ do not contradict "the olfaction hypothesis", but only a specific version that was published at that time^{5,6}.

Concerning the role of olfaction: (1) Gould writes that in my experiments the nerve-sectioned birds were "slower to home" than untreated pigeons. In fact, they did not home at all⁶. (2) It is well known that anosmic pigeons are not dis-

oriented, but that their preference for a particular compass direction persists⁶⁻⁸. When homing is concerned, however, the level of homeward orientation is crucial^{6,8}, and the parameters extracted by Gould (vector lengths 0.8 and 0.4, average deviation 38°) include no information on that. Homeward components of anosmic pigeons (initial bearings as well as recoveries) are drastically and significantly reduced^{6,9}.

(3) Gould quotes Keeton's mention of a few nasal-tube pigeons that had landed after a short flight¹⁰, but ignores the much more conclusive 75 recoveries of anosmic pigeons (and 61 of controls) at distances ranging from 10 to 700 km reported in my paper⁶. Differences between experimentals and controls in flight directions, yet not in distances, are highly significant. There is no "contrast to the disoriented birds in other" (which?) "experiments who continue gamely flying".

(4) In the nasal-tube experiments of Keeton *et al.*¹¹, "no disorientation was evident". This is true, but appropriate data analysis revealed significant differences between experimentals and controls in initial homeward orientation⁶, and this is the crucial point (see above). (5) Gould confuses the matter when he mixes the primary question of whether olfaction is a substantial component of pigeon homing with the secondary question of how it might be integrated into the navigational system. By considering one specific hypothesis⁵ about this secondary problem, and thus many experiments merely indirectly concerned with olfaction, he creates unjustified doubts also with respect to an affirmative answering of the primary question. After recent extensive discussion of the related problems⁶, this kind of review of the field is obsolete.

The examples selected may warn the reader against trusting this review. Related literature published in the meantime is quoted in ref. 12.

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IN his recent article on the homing mechanism of pigeons, Gould¹ says that when I replicated experiments with anosmic pigeons in Germany² no effect on initial orientation was evident, though homing speed was slower than in controls.

I would like to point out that, in my first series, pigeons made anosmic by inserting nasal tubes were homeward oriented and performed rather well in homing from a familiar site, whereas, when released from an unfamiliar site, they were randomly oriented, unlike the homeward-directed controls (the difference in homeward directedness was significant). To say that the homing speed of experimentals was slower is an overindulgent assessment of their performances; 12 out of 16 birds were lost (against 2 controls out of 18).

In my second series, both controls and experimentals underwent the section of one olfactory nerve and the plugging of one nostril (the contralateral in experimentals, the ipsilateral in controls). Despite this minimization of differences in treatment, homing performances were significantly worse in experimentals, with 61 birds out of 92 lost (against 36 controls out of 90). In this case no differences in initial orientation could be found for the simple reason that bearings of both controls and experimentals were randomly distributed. In this particular respect my experiments were inconclusive, whereas the rest of my results support the olfactory hypothesis.

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GOULD¹ writes that certain results obtained by my colleagues and myself, which support the olfactory hypothesis, "admit of simpler non-olfactory explanations". According to a first, "simpler" explanation, pigeons with nostrils plugged or olfactory nerves cut are slower in homing because of "a generalized, distracting trauma". Besides the fact that anosmia affects not only homing speed but also homing success²⁻⁴, Gould fails to mention other important findings. (1) Homing capacity is strongly reduced in anosmic birds as compared with controls subjected to the same trauma (one nerve cut, one nostril plugged^{5,6}). (2) Birds not subjected to trauma are indeed impaired in homing when prevented from smelling by nasal tubes^{2,5,6}, and disoriented after treatment of the olfactory mucosa with local anaesthetic, provided that smelling during transportation has been prevented⁷. (3)

Anosmic pigeons are greatly impaired in homing from unfamiliar sites, but are not "distracted" by trauma when released from familiar ones^{2,5,6,8}. Thus, anosmia affects navigation, not motivation or capacity for homing by using familiar landmarks⁴.

The rotated orientation of deflector-loft birds⁹⁻¹¹ is attributed by Gould to the rotation of polarized light and of other visual cues rather than to that of incoming wind (another "simpler" explanation). Unfortunately, he drastically misinterprets the findings of Waldvogel and Phillips¹²⁻¹⁴. As these authors showed, pigeons raised in special lofts, which rotated wind and visual cues in different directions, were strongly deflected in accordance with wind-borne cues (Gould has probably got mixed up between pigeons raised in the deflector-lofts and foreign birds kept in them for a short time).

Other experiments of our group, Gould states, "cannot be repeated" or "have proved difficult to replicate". However, among the replications of our experiments performed abroad there is at least one for each kind of experiment that was successful. Fiaschi and Wagner¹⁵ obtained positive results by applying masking odours to the beaks of pigeons, and the detour experiments of Schmidt-Koenig *et al.*¹⁶ were successful. In fact, when one pools the bearings that were expected to deflect to the left or right in all the tries, one obtains two distributions which are different ($P < 0.005$) and rotated as predicted.

Three more of Gould's statements must be challenged. (1) It is incorrect to say that homeward orientation in anosmic pigeons has never been abolished. When releases were performed avoiding the effect of directional biases, initial orientation did not turn out to be homeward or showed a very poor homeward tendency^{4,17,18}. (2) It makes no sense to say that we found that "homing speeds in the strong-odour experiment were unaffected", since untreated controls for comparing homing speeds were not used⁹. (3) It is not true that we found that "rearing birds with no olfactory experience whatsoever does not affect orientation". In the case²⁰ quoted by Gould, the experimentals were oriented randomly or in a direction different from that of home and controls.

Pigeon homing is a complicated, intriguing problem, but I do not feel that Gould's review contributed to its clarification. The part of his report concerning the olfactory theory appears to be more biased than one might honestly expect from the supporter of a rival hypothesis.

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GOULD REPLIES—Wallraff's unusual techniques, data analyses and conclusions have always been found difficult to interpret. My approach was to apply a more conventional analysis to such of his original data as could be extracted from his papers. As indicated in the review, this included correcting the data for release-site bias—the "correct" orientation of birds is that adopted by controls rather than the actual map bearing—and considering only that universally used measure, the vanishing bearings. Viewed in this light, Wallraff's data begin to make sense to me. Wallraff, however, prefers his own methods.

Benvenuti, in the abstract of the paper cited, states "the poor initial orientation in either controls or experimentals in many single experiments and in pooled data was an insufficient basis for evaluation of the influence of olfactory deprivation on homeward directness". In other words, there was no clear effect on initial orientation.

As indicated in the review, Papi's results generally could not be replicated by either Keeton or Schmidt-Koenig. Even a casual reading of the papers cited removes the force of Papi's present remarks—in no case do the experiments cited clearly support the olfactory hypothesis to even a mildly sceptical mind. This is not to say that odour may not play a part in homing.

My conclusion, then as now, after reviewing all the evidence, is that "the olfactory hypothesis, though initially very exciting, has lost some of its lustre as a result of these many problems. It may yet turn out to be true..." The interested reader should examine the original papers bearing in mind (for what they are worth)

the criticisms of the various methods and conclusions outlined in my review.

JAMES L. GOULD

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Incorrect colicin sequence

In response to an enquiry from Mr R. Parker of New Haven, Connecticut: In 1979, Dr D. H. Watson and I reported N-terminal and C-terminal amino acid sequences for colicin E1¹. In the same paper we also described a cell-envelope-associated proteolytic activity that cleaved colicins E1, E2, E3 and K specifically to yield fragments that had either biological 'killing' activity or cell-binding properties. We suggested that such proteolysis might normally function to facilitate the transport of a colicin fragment to its target site for killing.

Early in 1980, Dr S. Luria, at the Massachusetts Institute of Technology, informed me that the N-terminal data obtained by Dr Watson were not consistent with those obtained in his laboratory, and in the summer of 1980, I circulated a note to a number of laboratories working with bacteriocins and similar proteins, informing them of our questionable data. Subsequently, direct sequencing of the colicin E1 gene² has confirmed that our published sequence data are erroneous. Moreover, although cell-envelope-mediated proteolysis of colicins of the kind described in our report has been demonstrated by several laboratories, its specificity and role in normal colicin function are not proven by our experiments.

DAVID J. SHERRATT

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- Watson, D. H. & Sherratt, D. J. *Nature* **278**, 362-364 (1979).
- Yamada, M., Ebina, Y., Miyata, T., Nakazawa, T. & Nakazawa, A. *Proc. natn. Acad. Sci. U.S.A.* **79**, 2827-2831 (1982).

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 500 words and the briefest of replies, to the effect that a point is taken, should be considered.

BOOK REVIEWS

Fools and pedants

Fred Dainton

FIFTEEN years ago student unrest was widespread. Those of us who experienced it think of that period as a bygone age and speculate what those students, now in their mid-30s, are doing. But that is not the only question to be asked. More important are: Why was the movement in the United Kingdom so mild relative to those in Germany, France and the United States? Was it because it was managed better at the university level, that vice-chancellors are better than rectors and presidents at student relationships? Was it that the internal structures of the British universities and their interdigitation with central government were more adaptable, better able to absorb the assault on their values and to turn the anger into something more productive? And perhaps of greatest importance: What did this unrest imply for the prospects of universities in the future?

In so far as Britain is concerned the answer to at least one aspect of the last question is plain. As a former chairman of the University Grants Committee cast in the role of securing resources for universities, I can assert categorically that nothing made my task harder than the inanities and the behavioural extremes of those tiny groups of militant students and their unwillingness to allow free speech by others. They damaged the student image and with it the cause of university education.

Many books have been written about the causes of student unrest and the forms it took. By and large the techniques of "direct action" — the "sit-in", the attempts to give it legitimacy through the "general assembly", the trappings of a participatory democracy and so on — were common to all countries. So too were the characteristics of the activists, being drawn largely from the upper rather than the lower socio-economic groups and prone to fragment into doctrinally distinct groups — Maoists, Anarchists, Trotskyites, orthodox communists and those flirting with Laingian psychology or the drug subculture. The immediate causes seem to have differed from country to country; protest against the Vietnam war in the United States, nuclear power in Japan, the military-industrial complex generally.

This book is quite different. It is concerned principally with the way in which the universities and the governments in the United States and Europe reacted to student unrest. It was commissioned in

Universities, Politicians and Bureaucrats: Europe and the United States. Edited by Hans Daalder and Edward Shils. Pp.510. ISBN 0-521-23673-8. (Cambridge University Press: 1982.) £37.50, \$74.50.

1974 by the International Council on the Future of the University (ICFU), which had grown out of the concern of a group of individual academics who met in 1970. They were convinced that a crisis existed in universities and that some fundamental academic and social values were threatened because, at least in the view of some of ICFU, governments and universities in some countries were accommodating to the unrest to extents which compromised those values. ICFU therefore asked Hans Daalder, Professor of Political Science at the University of Leyden, to bring together a group of scholars of high reputation, each of whom would be able to write about a particular country with scholarly objectivity.

The result of the labours of this group, mainly political scientists or sociologists, together with a lawyer and an economist, is a series of interesting essays concluded by a masterly, succinct and lucid survey by Daalder himself. There is no anger; just calm and judicial appraisal of the evidence after it had been subjected to the scrutiny of real professionals. Once started on it, I found the book extremely difficult to put down. Even setting aside what I acknowledge must be a deep personal interest in the subject, I believe that it would make enjoyable and profitable reading for anyone who cares about universities and their role in society. Indeed, I would recommend it to all who work in universities whether as administrators or as teachers, scholars and researchers, and to bureaucrats and politicians whose decisions have such a powerful influence on universities, especially in Europe. Daalder apologizes for the delay in the publication of these essays, but, in my view, that delay is a positive advantage. Not only has it allowed the writers a better perspective, the making of judgements in a more dispassionate manner than would have been possible when the phenomena being examined were also being experienced, but also the book appears at a time when the world recession has caused a reordering of social priorities and, for the students, of their personal priorities. The time has now come, as Rutherford said in the 1930s, when "We

have less money, we must think harder".

The reaction in British universities to student revolt went through several phases. First there was the shock and hurt felt by academics that some students distrusted them and did not reciprocate the proffered friendship of the teacher. Then came annoyance at the disruption of work followed by resolve to identify and remove any genuine causes of grievance. Over the ensuing years adjustments were made at many levels within the universities but no vital principles were surrendered. Students were brought into governance and, like the professors, some made useful contributions and some did not; their verbosity was and remained in inverse relationship to the significance of their utterances. The essential academic freedoms of selection and assessment of students, of the hiring, firing and promotion of academic staff and of the control of the curriculum and how it should be presented were reserved as the exclusive responsibilities of the dons. Government did not intervene because it had no *locus standi* under the charter and statutes of each university and the most that it could have done would have been to persuade the Queen to revoke the Royal Charter or to instruct the University Grants Committee to withhold grants from a particular university. Either of these acts would have taken far too long to bring about and would have damaged the interests of the vast majority of students who were going about their business in the way that students have always done.

How very different was the situation in Europe, in some countries of which, unlike Britain, many universities were and are subject to special laws and professors were officials whose appointments were ultimately in the hands of the state. The disruption was greater, the student protest more violent and destructive, and there were many justifiable complaints about the manner in which many universities were run, which was more appropriate to the nineteenth century and quite unable to meet modern needs. In essence, professors were all powerful, running their institutes like private baronies; their assistants had little status and little influence in shaping the policies of either the institute to which they belonged or the university of which it formed a part. Because they did not share in this responsibility they felt no compelling reason to show loyalty to the institute or to the university when the

troubles began. With no limitations on the number of students entering, and with very long courses before graduation, student numbers were high and teaching was mainly didactic, with the formal lecture to extremely large audiences the predominant mode. There was little small-group teaching and virtually no tutorials and, therefore, little personal contact with the full professors until the student proceeded to the level of serious research and the preparation of a thesis. Reforms were certainly overdue in Germany, France, Italy, Belgium and Holland. These reforms seemed beyond the imaginative power of the academics, at least during crisis, and even if the reforms could have been devised they would have been likely to be beyond the legal power of the university to implement. Therefore the state had to intervene. The forms that this intervention took in different countries are described and assessed in the book.

The overriding impression these accounts give is one of general ineptness on the part of politicians and bureaucrats; in matters of university governance they overreacted and overshot the mark by a large margin. They did so in part because they themselves were unsure of what universities should be and in part because of a rather pervasive mood that participatory democracy was a recipe for harmony in all organizations. A typical result was the imposition of the *Dreigruppenprinzip* on universities by which the governing body was made equally tripartite between elected academics, elected non-academic staff and elected students. The result was predictable; interminable and inconclusive meetings and the obfuscation of the basic truth that university education, whether vocational or non-vocational, is a partnership between the old and experienced and the young and inexperienced in which the former put their knowledge and experience at the disposal of the latter in a way which draws out their talents of intellect and personality as well as providing mental discipline, knowledge and skills. There is a memorable passage in one of A.N. Whitehead's lectures delivered 55 years ago:

Youth is imaginative, and if the imagination be strengthened by discipline, this energy of imagination can in great measure be preserved through life. The tragedy of the world is that those who are imaginative have but slight experience, and those who are experienced have feeble imaginations. Fools act on imagination without knowledge, pedants act on knowledge without imagination. The task of a university is to weld together imagination and experience.

Readers of this book will classify the actors — the professors, the politicians and the bureaucrats — in their own way. They will not always agree with the perceptive comments on this brief but exciting episode in university history, but they will find much food for thought.

Sir Frederick Dainton is Chancellor of the University of Sheffield.

Bioenergetics: is there any demand for quantum mechanics?

Terrell L. Hill

Biology and Quantum Mechanics. By A.S. Davydov. Pp.229. ISBN 0-08-026392-5. (Pergamon: 1982.) \$45, £22.50.

AT THE end of the introductory chapter, A.S. Davydov states the theme of his book:

Undoubtedly the effective use of the chemical energy from food products by living organisms for muscle contraction, the production of concentration gradients, the conduction of nerve impulses, protein synthesis, etc., is associated with the excitation of special weakly relaxing degrees of freedom.

Davydov is referring here to excitons and solitons, especially in one-dimensional molecular systems (for example, the α -helix). If, however, he had used "conceivably" in the above quotation in place of "undoubtedly", he would have been on much firmer ground.

As it stands, most readers with some understanding of modern bioenergetics will be put off by some of the claims in the preface and first chapter. This is a pity because most of the book is, in fact, devoted to a review of assorted subjects in molecular biophysics and bioenergetics. Such a review could prove quite useful in providing a first glimpse of this field for a physical scientist. It is notoriously difficult for many physicists, especially theoreticians, to acquire the necessary background and perspective in order to move into biological research; a guide written by a distinguished physicist ought to ease the pain.

The potential reader should be aware, however, that the discussion of almost every individual topic stops well short of the present state of knowledge and understanding at the molecular level. The treatment of most subjects covered here is roughly five years out of date, that of muscle contraction models and theories even more so. A physical scientist who begins with this book would do well to turn next to the second edition of Lubert Stryer's *Biochemistry* (W.H. Freeman, 1981) to obtain a more thorough and current treatment of most of these topics.

To return to the initial criticism: conventional kinetic, thermodynamic and statistical mechanical concepts provide a completely adequate theoretical foundation, at the molecular level, for the understanding of all of the special biological topics treated in this book. This foundation has to be supplemented, of course, by still incomplete mechanistic and structural details in each individual case. It is not necessary to invoke any relatively exotic quantum mechanical explanations, as the author does (for example, for muscle contraction). This is certainly not to say that such approaches are untenable:

rather, elementary and straightforward explanations should be retained and tested until there is evidence that more complicated theories are a necessity. There is no such need in bioenergetics at the present time.

Terrell L. Hill is at the National Institutes of Health, Bethesda, Maryland.

A class apart

William Hodos

Brain and Intelligence in Vertebrates. By E.M. Macphail. Pp.423. Hbk ISBN 0-19-854550-9; pbk ISBN 0-19-854551-7. (Oxford University Press: 1982.) Hbk £20, \$29.50; pbk £10.95, \$14.95.

THE histories of comparative studies of brain anatomy and of intelligence in vertebrates have followed parallel, but for the most part independent courses during the past century. Each began with anecdote, followed by a slow accumulation of hard facts. The past two decades have seen a dramatic increase in the amount of data generated in both disciplines and breaks with theoretical tradition.

Euan Macphail's book is an attempt to achieve a fusion of these two fields. Although written with considerable clarity, much of the book does not lend itself to effortless reading. Nonetheless, the exertions of the reader will be amply rewarded by an understanding of the salient issues in the brain-intelligence field and by an awareness of the fallacies, gaps in knowledge and intellectual pitfalls that have bedevilled it since its inception.

Apart from the introductory and concluding sections, each chapter is devoted to a single class of vertebrate and has the same general organization. First, the evolutionary history of the class is briefly reviewed followed by a short account of the taxonomic organization within the class. Next, the author presents a description of the anatomy of the forebrain of some individual species characteristic of the class, before turning to a critical survey of the various types of learning procedures that have been attempted with members of the class. Each chapter ends with a summary and conclusions.

The two introductory chapters discuss the measurement of intelligence in animals and human beings, offer a classification of learning tasks, summarize the evolution and classification of vertebrates and review the literature on general morphological brain indices. The two concluding

chapters, which deal with the relationship between language and intelligence in human beings and the evolution of the brain and intelligence, include an analysis and critique of the studies of language and communication in non-human animals and their relationship to human and animal intelligence.

This final section also contains the author's evaluation of the current state of knowledge of comparative studies of the brain and intelligence, which he characterizes in two working hypotheses to be tested by future research. The first is that, with the exception of human beings, neither qualitative nor quantitative differences in intelligence exist between vertebrate species; the second, that the capacity for language so affects the human's approach to problems of all kinds that the result is a qualitative difference in intelligence between humans and non-human vertebrates. Moreover, the influence of language is so pervasive in human reasoning that one may be unable to assess the role of non-linguistic reasoning in humans for comparison with non-human animal intelligence. These hypotheses are refreshing departures from conventional wisdom and will be the basis for considerable discussion.

While there is much to commend in the book, the treatment of certain topics falls short of perfection. For example, the only region of the brain that is discussed consistently is the forebrain and then usually only the telencephalon. Macphail justifies this emphasis by pointing out that it is this region that appears to undergo the greatest growth and development in evolution. Although his point is well taken, critics could argue that the telencephalon does not function in isolation and has important connections with the diencephalon and mesencephalon; moreover, to ignore the role of the mesencephalon in processing information about the spatial properties of the external environment may be to disregard an important aspect of the brain-intelligence problem.

Another area that may disappoint some readers is Macphail's limitation of his account to certain types of formal laboratory studies of learning. Phenomena such as tool-use and learning by imitation are not discussed, the vast ethological literature and its implications for assessing the nature of animal intelligence being specifically excluded. Macphail makes a case for the latter omission, but I suspect that readers with an ethological bent may not be fully convinced.

Such disappointment as the reader might feel will nonetheless be soothed by the wealth of well-organized information, original interpretations and challenging ideas presented in this volume. I recommend it highly to anyone with a serious interest in the brain and intelligence. □

William Hodos is a Professor of Psychology at the University of Maryland, College Park.

View of Kew from the inside

S.M. Walters

Kew: Gardens for Science and Pleasure. Edited by F.N. Hepper. Pp.195. ISBN 0-11-241181-9. (HMSO/Stemmer House: 1982.) £9.95, \$24.95.

THIS handsome and lavishly illustrated book consists of a series of essays by sixteen past and present members of the staff of the Royal Botanic Gardens under the editorship of Nigel Hepper, himself a senior taxonomist in the Kew herbarium. The subject-matter covers all three parts of the Kew empire, namely: the public gardens themselves; the herbarium and other research institutions not normally visited by the public; and Wakehurst Place, the magnificent "satellite" garden in Sussex, which became part of Kew in 1965.

The introduction explains that the essays "are intended to convey a broad view of Kew's history and the horticultural and scientific work that have given the Gardens their world-wide reputation". Ray Desmond, formerly Chief Librarian at Kew, contributes the first chapter, which sketches the history of the Royal Gardens, and the remaining essays are grouped into three sections: "Gardens for Pleasure" (3 chapters); "Living Botanical Collections" (9 chapters); and "Botanical Research"

(7 chapters). The decision to integrate Wakehurst with the "old Kew" by devoting chapters to the new garden in each of the main sections, though logical, has not been entirely successful; in particular, the description of the memorial garden to Sir Henry Price, last private owner of Wakehurst, seems out of place in the first section.

A welcome feature of the book is the treatment of aspects of Kew as a botanical research establishment. Chapter 17, devoted to multidisciplinary studies of *Crocus*, represents a brave attempt by no fewer than five staff members to explain at a popular level mysteries such as cytotoxicology: fortunately, the genus lends itself to attractive colour photography! Another important chapter deals with Kew's new, explicit conservation role, the most urgent of all its tasks in face of the threat to the world's flora.

Reading the essays produces a strong impression that the authors are all loyal Kew people. A measure of how parochial their view is can be obtained by looking up other botanical gardens in the index: for example, Edinburgh, by any standards one of the world's most important scientific gardens, gets only a single, oblique reference, on page 31. Even in those few areas where the outside world has dared to question the morality of Kew's activities — such as the manner of acquisition of stocks of rubber plants in South America a century ago — the loyal Kew man says only (p.131): "... popular accounts indicating that Wickham dishonestly smuggled out the *Hevea* seeds are unfair both to the collector and to Kew". At least a strong antidote to such sycophantic writing is available (if in very extreme form) in the recent study by Lucile Brockway (1979) cited in the select bibliography at the back of the volume. Kew still awaits a good, definitive study, preferably by a reasonably sympathetic and well-informed outsider, who can ask questions about policy and aims such as are hardly touched on in this work, even in the interesting final essay on "Kew in the Future" contributed by the retired Director, Professor Brenan.

The world of book publishing presents increasingly baffling questions to the layman. Just at the time when we are told that Her Majesty's Stationery Office will no longer subsidize official publications of any kind, that same Office publishes such a lavish, popular book as this at a price which implies a large subsidy or great optimism and an enormous print run. Whatever the explanation, we can only express our appreciation, and wish the venture success.

S.M. Walters is Director of the University Botanic Garden, Cambridge, and author of The Shaping of Cambridge Botany (Cambridge University Press, 1981).

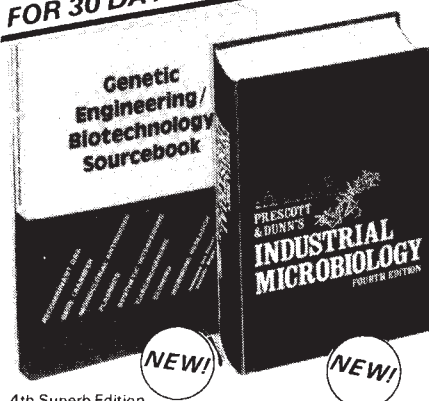
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A few fruits from a large orchard

John B. Furness

Biology of Serotonergic Transmission.
Edited by Neville N. Osborne. Pp.522.
ISBN 0-471-10032-3. (Wiley: 1982.) £30, \$69.

IN MAN, serotonin (5-hydroxytryptamine) nerves have been implicated in the control of various functions, including sleep, temperature regulation, pain and the secretion of pituitary hormones. At the other extreme, serotonin nerves in annelids and molluscs have been extensively studied because their prominent cell bodies can be identified by size and position in any individual animal. Serotonin is also found in other invertebrates, and, in the various vertebrate classes, is present in both the CNS and peripheral (intestinal) nerves.

The declared purpose of this book is to provide an up-to-date report of the state of serotonin research in the 1980s and, to a large extent, the various authors have done well by the topics covered in their individual chapters. However, in that the book only partly covers the field, Dr Osborne might well have referred his readers to other books and reviews - for example to the massive five-part work *Serotonin in Health and Disease*, edited by W.B. Essman and published by Spectrum Publications in 1978-1979.

Broadly, the articles in the present volume can be placed in one of two groups - those dealing with the (mainly biochemical) properties of serotonin neurones and those dealing with serotonin neurones in particular organs, species or life stages.

In the first group there is excellent coverage of the biosynthesis of serotonin, its storage and release, axonal transport, turnover, and uptake into axons and storage vesicles. Except for the chapter on axonal transport, which also deals with molluscs and makes occasional comparisons with platelets, these contributions are concerned with the properties of serotonin neurones in the CNS of mammals, principally the rat. This imbalance is only partly redressed in the final chapter of the book where some of the biochemical characteristics of serotonin neurones in gastropods are described.

Included in the second group is a succinct but commendably clear summary of the arrangement within the mammalian CNS of serotonin nerve cells and their processes. Another chapter documents the use of radioactive ligand-binding studies to distinguish between 5-HT₁ and 5-HT₂ receptor types in the CNS. These studies

may eventually provide a basis for interpreting differences in the behavioural effects of drugs acting at serotonin receptors. Possible roles of 5-HT in the mammalian central nervous system are dealt within a group of three further contributions; this is one of the weak parts of the book because so much is left out. The first summarizes the putative roles of serotonin but is disappointingly brief. And only two aspects of serotonin's central roles are dealt with at length in the other two chapters, namely serotonin and learning, and serotonin involvement in dyskinesias (the latter subject also appears at the end of a separate article on serotonin neurotoxins).

Serotonin nerves of the mammalian gastrointestinal tract (with some reference to other classes of animal) are reviewed next. As the existence of serotonin nerves in the gastrointestinal tract has been difficult to establish, the author gives proper emphasis to evaluation of the evidence for their presence. Another subject of some controversy, namely the proposed presence of serotonin neurones in the vertebrate retina, is covered in the following contribution. Finally, two chapters give lucid accounts of current research on serotonin neurones in leeches and gastropods.

Perhaps more than anything else in this field, a comprehensive and critical review of the pharmacology of serotonin receptors is needed. Yet in the only chapter explicitly dealing with drugs acting on serotonergic neuronal systems, a mere two pages and a table are given to a disjointed discussion of the subject. Not only is there a failure to do justice to receptor classification, but the very real problems of the additional actions of serotonin antagonists are overlooked; for example, the agonist actions of methysergide and LSD are not mentioned, nor is the anti-histaminic action of cyproheptadine. Most of the "non-specific" actions of other drugs acting on serotonin systems are also ignored.

Patchy coverage and lack of cohesiveness are common in edited works of this sort. But here they have reached such a pitch - inhibitors of the synthesis of serotonin are dealt with in four separate articles without cross-referencing, for example - that most of the contributions might just as well be in review journals. In mitigation there are some excellent chapters which are valuable works of reference in their own right. However for only a part of its potential audience will these chapters justify purchase of the entire book. □

Paper Fuller

Macmillan, New York/Collier Macmillan have issued a paperback edition of R. Buckminster Fuller's *Synergetics: Explorations in the Geometry of Thinking*, first published in 1975. The new edition incorporates revised illustrations, and costs \$12.95, £9.95.

John Furness is Associate Professor in the Centre for Neuroscience and the Department of Human Morphology at Flinders University, South Australia.

BOOKS RECEIVED

Mathematics

- BAXTER, R.J. Exactly Solved Models in Statistical Mechanics. Pp.486. ISBN 0-12-083180-5. (Academic: 1982.) £43.60, \$89.50.
- EHRENBORG, A.S.C. A Primer in Data Reduction: An Introductory Statistics Textbook. Pp.305. Hbk ISBN 0-471-10134-6; pbk ISBN 0-471-10135-4. (Wiley: 1982.) Hbk £22.20, \$49.95; pbk £6.95, \$15.75.
- LAPIDUS, L. and PINDER, G.F. Numerical Solution of Partial Differential Equations in Science and Engineering. Pp.677. ISBN 0-471-09866-3. (Wiley: 1982.) £35, \$59.75.
- PHILIPS, J.L. Jr. Statistical Thinking: A Structural Approach, 2nd Edn. Pp.181. Hbk ISBN 0-7167-1379-9; pbk ISBN 0-7167-1380-2. (W.H. Freeman: 1982.) Hbk £10.50; pbk £4.95.
- RUSTAGI, J.S. and WOLFE, D.A. (eds). Teaching of Statistics and Statistical Consulting. Pp.548. ISBN 0-12-604540-2. (Academic: 1982.) £36.
- WELKOWITZ, J., EWEN, R.B. and COHEN, J. Introductory Statistics for the Behavioural Sciences, 3rd Edn. Pp.369. ISBN 0-12-743270-1. (Academic: 1982.) \$19.75.

Astronomy

- BRUCK, H.A., COYNE, G.V. and LONGAIR, M.S. (eds). Astrophysical Cosmology. Pontificia Academiae Scientiarum Scripta Varia, 48. Proceedings of the Study Week on Cosmology and Fundamental Physics held September–October 1981. Pp.600. (Pontificia Academiae Scientiarum: 1982.) Np.
- FRIEDLANDER, A.L. *et al.* (eds). Astrodynamics 1981, Vol. 46. Part 1: Advances in the Astronautical Sciences. Proceedings of the AAS/AIAA Astrodynamics Conference held August 1981, Nevada. Pp.531. Hbk ISBN 0-87703-159-2; pbk ISBN 0-87703-160-6. (American Astronautical Society: 1982.) Hbk \$55; pbk \$45.
- FRIEDLANDER, A.L. *et al.* (eds). Astrodynamics 1981, Vol. 46. Part 2: Advances in the Astronautical Sciences. Proceedings of the AAS/AIAA Astrodynamics Conference held August 1981, Nevada. Pp.535-1088. Hbk ISBN 0-87703-161-4; pbk ISBN 0-87703-162-2. (American Astronautical Society: 1982.) Hbk \$55; pbk \$45.

Physics

- BASOV, N.G. (ed.). The Dissipation of Electromagnetic Waves in Plasmas, Vol. 92. Pp.101. ISBN 0-306-10969-7. (Plenum: 1982.) \$49.50.
- BUTTON, K.J. (ed.). Infrared and Millimeter Waves, Vol. 5: Coherent Sources and Applications, Part 1. Pp.343. ISBN 0-12-147705-3. (Academic: 1982.) \$52.
- DAVID, P. *et al.* (eds). Dynamics of Nuclear Fission and Related Collective Phenomena. Proceedings of the International Symposium held October 1981, Germany. Lecture Notes in Physics, Vol. 158. Pp.462. Pbk ISBN 3-540-11548-X/0-387-11548-X. (Springer-Verlag: 1982.) DM52, \$23.10.
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